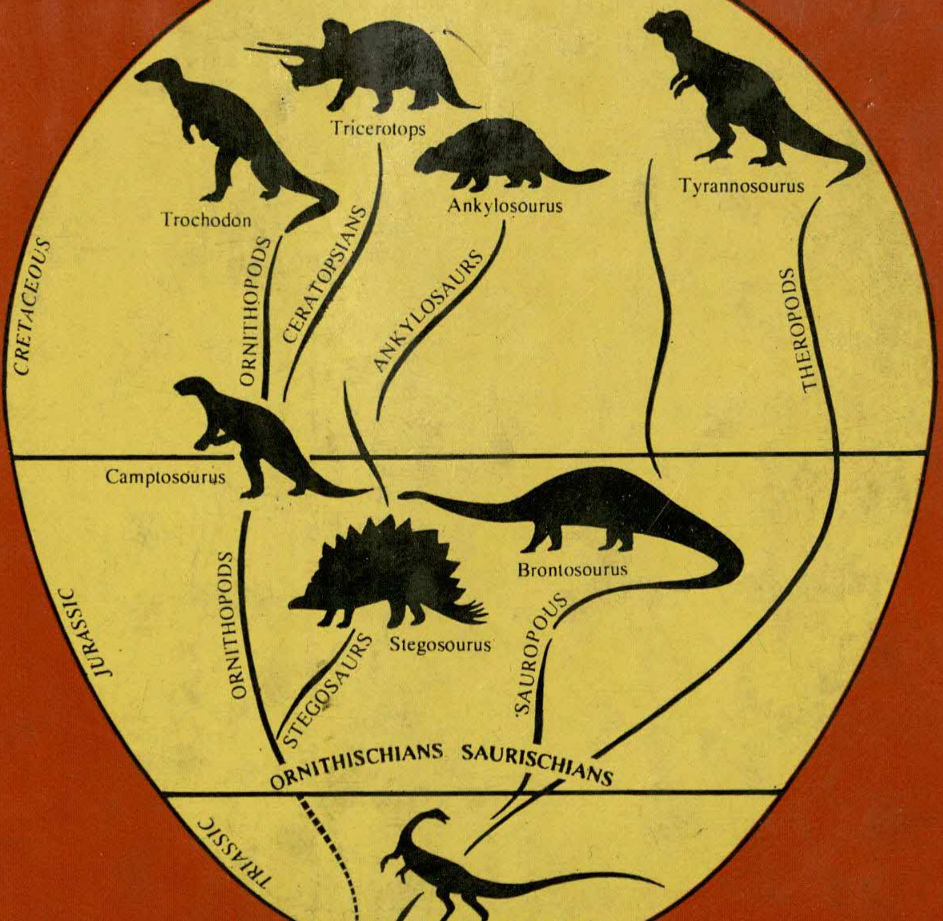
PERSPECTIVES IN ZOOLOGY

Volume 1

Editors

Bhoomittra Dev
Rameshwar Singh
U.D.Sharma



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CBS PUBLISHERS & DISTRIBUTORS
485, Jain Bhawan, Bhola Nath Nagar, Shahdara, Delhi-32

First Published in 1989

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Printed in India

Acc no- 16714

Published by Satish Kumar Jain for CBS Publishers & Distributors
485, Jain Bhawan, Bhola Nath Nagar, Shahdara, Delhi-110032, (INDIA)

Printed at : Army Printing Press, 33, Nehru Road, Sadar Cantt., Lucknow.

PREFACE

Though a large number of text-books and journals are available, there is an acute need of authentic and concise information about certain topics, especially selected for our undergraduate, post-graduate, research and other students. Keeping this in view, publication of "Perspectives in Zoology" was envisaged. More than half of the topics deal with physiology and cell, biology, *e.g.* physiology of neuron and muscle, aging, colour physiology and protein synthesis. Chapters on plant nematodes and microbes on one side, and dinosaurs and ecology on the other, have been appended to make the area of interest more broad-based.

It is heartening to mention that some of the top-most scientists have contributed their articles for publication. That it has been delayed owing to reasons beyond our control is regretted.

We hope that the publication will fulfill a long-standing need. We are looking forward to suggestions for selecting additional topics in future. Any other critical comments are most welcome so that we may serve as a better medium between the erudite authors and keen and alert readers.

We desire to thank Dr. A.K. Sharma, Shri S.M. Singh and Shri Sanjive Shukla for their invaluable help in its publication.

30.9.88

Bhoomittra Dev
Rameshwar Singh
U.D. Sharma

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1

THE MECHANISM OF MUSCULAR CONTRACTION

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Introduction

It is currently believed by many biologists that the crossbridge model of muscular contraction [1–4] has resolved the problem of elucidating the basic mechanism of contraction in principle and only a few details remain to be filled in. While the text books depict the process of tension generation with convincingly elaborate figures at the molecular level, the situation is becoming increasingly uncomfortable and doubtful for those who are engaged in active research in this field. Though it is unquestionably true that during shortening of muscle the thin filaments slide into the interlattice spaces of the thick filaments, the aspect of the crossbridge model which is becoming more and more uncertain is the mechanical role played by the crossbridges. These are supposed to get attached to the actins of the thin filaments and cause this complex to undergo mechanical transformations in a manner that results in the generation of tension across the thick and the thin filaments. Each "power stroke" is energized by the free energy released by the hydrolysis of one ATP molecule. The model considers crossbridges as molecular muscles which extend out from the thick filaments, attach at specific sites on G–actins and pull the thin filaments, to bring about the relative sliding. It is, therefore, a natural expectation that the contractility of these molecular muscles would manifest itself in some deformation (conformational changes) in some part of their structure and/or a change in their angles. The finding[5] that the crossbridges do bind to the actins in the state of rigor in an "arrow head" configuration at a specific angle was, therefore, very encouraging. However, all later efforts have failed to detect any second state of the acto-crossbridge complex.[6–8] There is neither any substantial change in the attitude of the crossbridge nor any global conformational change detectable during its interaction with the actins. All that is seen, *i.e.* deduced from the studies of the structural changes during contraction, is an outward movement of the crossbridges. This seems to occur, however not only in the region of overlap of myofilaments but also in the H-band and continues for several seconds after the contraction is over.[9] In view of the proven sensitivities of the techniques which have been employed, there is little room for doubt that the crossbridges cannot perform the role of mechanical force generators ascribed to them by the crossbridge model.

The crisis for the crossbridge model lies less in the lack of supporting evidences mentioned above than in the very fundamental thermodynamic problem : the occurence of "unexplained energy"[10]. In all muscles, under all conditions there is a substantial release of energy (heat+work) during contraction which cannot be accounted for by the splitting of phosphocreatine during the same period. After the contraction is over the same amount of chemical energy as was missing during contraction is spent by the muscle without being used up in any known metabolic process. Obviously no model of muscular contraction can have any credibility if it cannot explain this particular observation. Indeed it is required of a thermodynamically tenable model to provide for the storage of chemical energy into some intermediate form which is released as heat and work during contraction and can be replenished during recovery. However, there is no scope for the storage of chemical energy in the crossbridge model where the transduction of chemical form of energy into mechanical is direct. Each cyclical interaction of the crossbridges with the actins is related to the steps of ATP hydrolysis in a one to one relation and hence the model envisages a perfect synchrony between the expenditure of chemical fuel and the release of energy by muscle.

The obvious conclusion to be drawn from the above discussion is the need for exploring new approaches to the contraction phenomenon. There are a number of biodynamic phenomena–contractility and motility in muscle and non–muscle cells, cytoplasmic streaming, mitosis and others–which are intimately related to each other. All these research areas offer great scope for fruitful investigation from new angles which were being ignored due to the euphoria arising from the initial successes of the crossbridge model.

In this article a new approach to the mechanism of muscular contraction [11–14] will be reviewed and extended. Emphasis has been laid on the fundamental aspects of the new approach. Mathematical derivations and quantitative results which have been already published have been left out to a great extent. For the sake of completeness a few sections are devoted to the basic information on structure, function and biochemistry. This is followed by a discussion of the possible mode of release of free energy of

ATP hydrolysis by myosin ATPase consisting in the accumulation of H$^+$ in the myosin heads. After a description of proton conduction properties in the hydrogen-bonded chains of proteins the reader is introduced to the new phenomenon in electrochemistry: proton-flux-mediated electroosmosis. This consists in the generation of active fluid streaming along filamentous macromolecules when the cationic diffuse layer around them moves under the action of a diffusion potential. The latter itself is generated by the flux of H$^+$ when an ATPase delivers H$^+$ into the hydrogen-bonded chains of the filaments. Contraction process is explained in terms of these results by utilizing the viscous drag of the active streaming on myofilaments as the force couple which causes the filaments to slide against an external force. In the Discussion a few important aspects of the contraction phenomenon are dealt with and some suggestions have been made regarding the extension of this approach to other biodynamic phenomena.

Fine Structure of Skeletal Muscle

Because of their precise organisation and easy handling, skeletal muscles have been used for the detailed studies of the structure–function relationship in preference to other muscle types. As such, theoretical models have been also built on the basis of the information available on this muscle. It is, however, by no means certain that it is more judicious to try to understand contractility at hand of this particular, possibly fortuitous, organisation of the contractile apparatus. Smooth muscles[15] do not have a regular filament lattice and contractile nonmuscle cells[16] do not have myosin organized in the form of filaments, the way these are found in skeletal muscle. Though we are forced to choose this muscle type because of existing, detailed information available on it, the reader is advised to note that there are lots of variations in the structure–function relationships and the attention should be focussed on the "common denominator" for all contractile machines found in the living organisms.

Individual skeletal muscle fibers[17],[18] are long, cylindrical, multinucleated cells attached to the tendons at their ends. Nerve fibers, blood, and lymphatic fluids are conveyed to these fibers through the connective tissues separating them. Each fiber is enclosed in its plasma membrane (sarcolemma) which is further enveloped by the basal membrane. The bulk of the fiber (80% by volume) contains the contractile apparatus. This is a collection of longitudinally arranged myofibrils extending over the whole length of the fiber. At their ends myofibrils have specialized structures for transmitting the contractile force to the tendons. Nuclei and mitochondria are arranged peripherally to the contractile apparatus. Around each myofibril the endoplasmic reticulum (sarcoplasmic reticulum, SR) forms a lace–like network in the form of tubules, sacs and fenestrated collars. Another membranous system, the transverse tubule system (T–system) proliferates into the interfibrillar spaces transversely from the fiber surface. The

T–system makes specialized contacts with SR at specific locations with respect to the band structure of the fibrils. The band structure of the fibrils and, since these are in register in the fiber, the striation of skeletal muscle fibers, arises from a very regular arrangement of two sets of filaments and their supporting structures as shown schematically in Fig. 1.

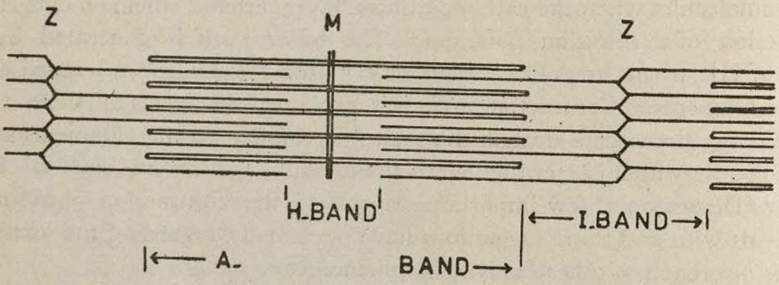


Fig. 1. Schematic representation of the arrangement of the thick and thin filaments in a myofibril of striated muscle.

One set of these myofilaments projects out from a dense, disc-like structure, the Z-disc, in two, longitudinally opposite directions. These filaments are about 1 micrometer long and have a diameter of about 8 nanometer. These are called the thin filaments or I-filaments. About 1.6 micrometer long and of a diameter of 15 nanometer, the filaments of the second set, called the thick (or A–) filaments, are organized in the form of a hexagonal lattice. The thick filaments are held at their centers in the regular lattice pattern by a network of structural proteins in the transverse plane. The latter gives rise to a dense line, called the M–line, in the middle of two successive Z–discs. The structural unit of a myofibril between two Z–discs is called a sarcomere. When the length of a sarcomere is greater than the length of the thick filaments, three bands are visible : the H–, A– and I–bands. The H–band at the centre of the sarcomere contains only the A–filaments and the the I–bands close to the Z–discs have only thin filaments. The A–band corresponds to the whole length of the thick filaments. In the region between the central H–band and one I–band the two sets of filaments interdigitate. In this region of overlap a transverse view shows each thick filament surrounded by six thin filaments in a ratio of one to two, the pattern being the sum of the patterns of the H– and I–bands as shown in Fig. 2.

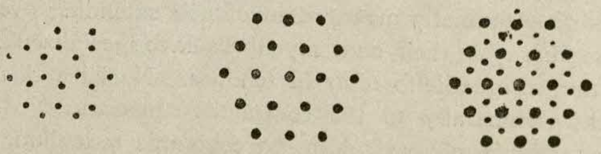


Fig. 2. The cross-sectional pattern of myofilaments in the overlap region (right) is the sum of those from the I-band (left) and the H-band (centre). The center to center distance between the thick filaments is about 40 nm.

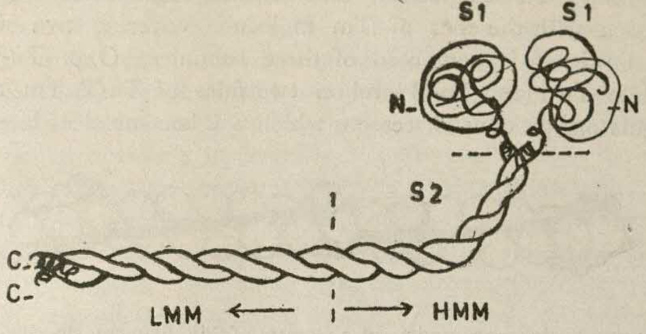


Fig. 3. Schematic representation of a myosin moleculer S2 and the two globular heads (S1) form a crossbridge. The tail part is about 90 nm long and S2 is about 50 nm long.

Thick filaments are composed of myosin molecules. As shown in Fig. 3, myosin is formed by two strands of identical polypeptide chains wound around each other in a rod portion (tail) and uncoiled towards the N–terminus in the form of two globular heads. The segment connecting the heads with the tail forms a flexible link. These parts can be separated by certain proteolytic enzymes and are then classified as light meromyosin (LMM, the tail), heavy meromyosin subfragment 1 (S 1 of HMM, head) and heavy meromyosin subfragment 2 (S 2 of HMM, the flexible link). In the thick filaments myosin molecules are arranged in an antiparallel way (Fig. 4), their tails pointing towards the M–line. A head–free region is formed at the centre of the filaments which gives rise to the pseudo H–band. The centres of the filaments are integrated into the M–line by means of a C–protein. The two globular heads together with the fiexible link constitute the crossbridges. The crossbridges are helically arranged in the thick filaments with a 42.9 nm axial repeat. Three crossbridges at every 14.3 nm, rotated by 40^0 over this interval is a possible and commonly accepted configuration.

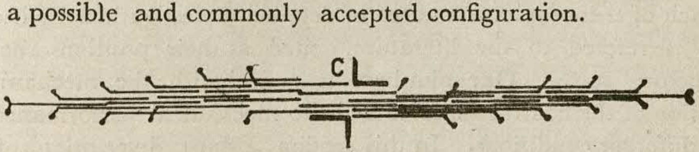


Fig. 4. A tail to tail antiparallel aggregation of myosin molecules is supposed to form the thick filaments in the skeletal muscle. In the central part of the filament, C-proteins connect the thick filaments to the structural complex of the M-line.

Thin filaments contain three proteins : G–actin, Tropomyosin (Tm) and Troponin (Tn). The globular G–actin monomers are polymerized in F–actin which comprises two strands of G–actins wound helically with a structural repeat of 74 nm, each turn having 13 G–actins, (Fig. 5). Tm are rod-shaped moleclues, about 40nm long and 2 nm in diameter. Two Tm molecules lie along the F–actin on opposite sides close to the grooves, each having contacts with 7 G–actins. Tn are globular and occur at regular intervals of 39 nm in association with the ends of Tm molecules covering two G–actins of F-actin. Tn itself is composed of three subunits. One, Tn-T binds to a Tm, while Tn-I and Tn-C bind on two sides of Tn-T. Tm and Tn are called regulatory proteins for reasons which will become clear later.

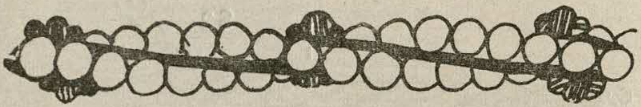


Fig. 5. Diagrammatic representation of a segment of thin filament showing two tropomyosin molecules (blackened) and three pairs of troponins (shaded) in association with the double strand of F-actin. Each long Tm contacts seven globular actin monomers and carries one Tn molecule at one end.

Functional Features of Skeletal Muscle

The process of muscle contraction may be described in terms of the sequence of the following events:

1. Excitation of the sarcolemma at the neuro-muscular junction by neuronal signals,[19]
2. Spread of excitation in the T-system by means of propagated action potentials,[20]
3. Release of Ca^{2+} from the terminal cisternae of SR,[21]
4. Interaction of Ca^{2+} with the regulatory proteins of the contractile apparatus[22],
5. Generation of muscular tension,
6. Removal of Ca^{2+} and relaxation[21].

Each of these topics is an area of detailed investigations by itself and the reader is refered to the literature, cited at their positions above, for an introductory study. Our aim here is to deal with the mechanism of the generation of the contractile force and the mechanical performance of muscle under different conditions. In this section a short description of the gross functional features of skeletal muscle will be given[23,24].

The mechanical response of muscle depends basically on three factors. These are, the type of stimulation that is given; the initial length of the muscle and the magnitude of its variation; and, lastly, the magnitude of the external load, P. A single action potential elicits a short duration response, the twitch. If repeated stimulation is given at short intervals there is a summation of contractile response in terms of tension averaged over a time period greater than the period of stimulation. As the stimulation frequency is increased one finally gets a sustained constant value of tension which is termed as tetanic contraction. Though stimulation leads to the development of muscular tension, it is the external load which determines whether it will shorten, stretch or maintain its length. An isometric contraction, *i.e.*, contraction without an accompanied change in the length of muscle, is obtained when the load is just equal to the tension developed by muscle. Lower and higher loads cause a shortening and stretching of muscle. At maximal stimulation, *i.e.*, when muscle is undergoing tetanic contraction, the maximum load it can hold without getting stretched is denoted by P_0 and the corresponding muscular force is called the isometric tension (T_0). Its value is about four times greater than the peak value of the force generated at constant length during a twitch. The value of P_0 lies around 20 Newton per square centimeter of cross-sectional area of muscle. However, this value as well as the contractile force in general depend on the length of muscle when it contracts. Maximum tensions are generated at the slack length of muscle. The value of tensions generated by muscle drop when the length at which it contracts is chosen to be appreciably larger or smaller than the slack length.

When the load on a muscle is kept less than P_0 and it is fully stimulated the generation of tension leads to shortening of muscle. This situation is termed as isotonic contraction. The velocity of shortening increases as successively lower loads are attached to the muscle. At $P=O$ one gets the maximum velocity of shortening, which is fairly independent of the initial length of the muscle around the slack length. Its value is typically about 6 cm/sec.

The tensions generated during stretching of muscle at loads $P > P_0$ are greater then isometric tension T_0. There is sharp rise in tension with increasing value of excess load. In other words, load–velocity curve around P_0 is not the same for $P > P_0$ as for the values of P, $P < P_0$ (see Eq. 1 below).

The mechanical events described above are obviously accompanied by the performance of work and heat generation in the muscle. During isometric contraction no work is performed since the load is not shifted from its position. Only a certain amount of heat is produced at a particular rate which is about 15 mJ per second per gram of muscle. It was found by Fenn[25] that when a muscle is allowed to shorten, it not only performs work but also generate more heat than it does during isometric contraction over the same period. Expressed more generally one can state the Fenn effect as follows : The total energy released by a muscle in terms of work and heat increases with the capability of the muscle to perform work against the external force. Thus there is some in-built feedback mechanism in muscle which regulates its energy output as a function of load.

A.V. Hill performed meticulous experiments on the generation of heat and performance of work in muscles and concluded,[26] that the generation of extra heat during shortening was proprotional to the velocity of shortening. He, therefore, came to the quantitative result :

$$v = b \frac{P_0 - P}{a + P} \qquad (1)$$

where v is the velocity of shortening and a and b are constants. This empirical equation describes the dependence of velocity on load fairly well for small velocity of shortening but not for v becoming negative and v approaching the maximum velocity (P=O). Hill revised [27],[28] the quantitative form of Fenn effect for large velocities by his further studies. It seems that the muscle shows a decrease in the release of energy output when the load is very small. The feedback mechanism in muscle takes care to avoid unnecessary wastage of energy also !

Biochemistry of Muscle Contraction

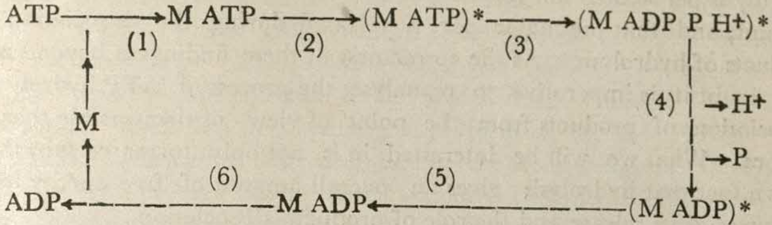
Although ATP was discovered in muscle it took several decades to establish that it was indeed the direct source of energy for muscular activity. (For the historical development of muscle physiology the reader is recommended to read Dorothy Needham's book, "Machina Carnis"[29]). As it happened there was no detectable change in the concentration of ATP during contractions. A. V. Hill's "Challenge to the Biochemists" (to prove that ATP was indeed the immediate source of chemical energy) was met only in 1962 by the discovery[30] that dinitrofluorobenzene (DNFB) can block the activity of creatine phosphokinase (CPK, also called creatine phosphotransferase, CPT). This enzyme catalyses the Lohmann's reaction :

$$ADP^{-3} + PCr^{2-} + H^{+} \rightleftharpoons ATP^{4-} + Cr$$

which runs with such a precise synchrony with the hydrolysis of ATP in muscle that there in no measureable decline in the level of ATP during a normal contraction. Curiously enough PCr is generated in muscles by the reversal of the above reaction outside of the myofibrils where the mass-action ratio is tilted in favour of the backward reaction. The concentrations of ATP and PCr in muscle are about 5 and 30 micromoles per gram of muscle respectively, the former being sufficient for only a few twitches. It is a question worth asking why should not have just a high level of ATP alone and do away with PCr?

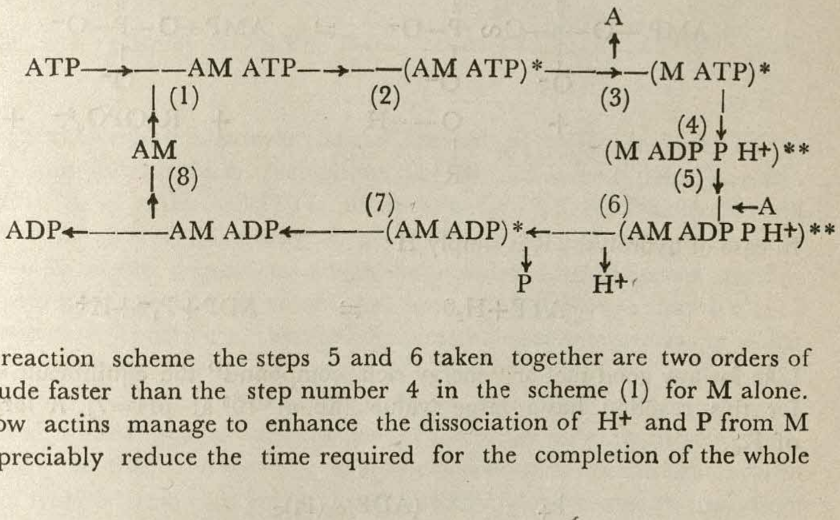
In a sarcomere, the capability to hydrolyse ATP is possessed only by the subfragment 1 of HMM. Each of the two globular heads of myosin can hydrolyse ATP without any positive or negative interference from the other. The rate of ATP hydrolysis by SI varies with time. The initial rate is very high but drops to a very low steady value after the hydrolysis of a few molecules of ATP. It is the second, steady state rate which is enhanced by the presence of actins and which makes the study of actomyosin ATPase a complex and challenging subject. Myosin ATPase (M) alone as well as together with actins in the form of F-actin and thin filaments is probably the best studied ATPase today. The results of several investigations have come up with the following schemes for the steps involved in a total cycle of ATP hydrolysis[31] :

(1) Myosin ATPase alone :



The asterisk indicate different states of the complexes formed by M with the substrate and products. The rate limiting step in this cyclic process is the dissociation of H^+ and phosphate from the enzyme product complex, i.e., step (4).

(2) Actomyosin ATPase :



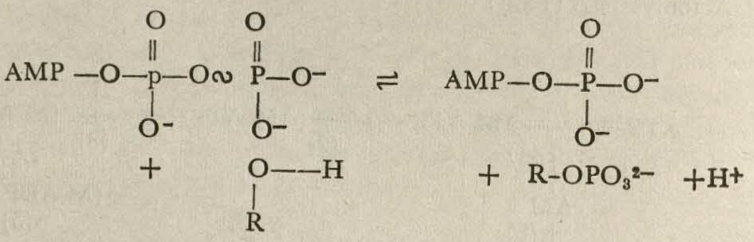
In this reaction scheme the steps 5 and 6 taken together are two orders of magnitude faster than the step number 4 in the scheme (1) for M alone. Somehow actins manage to enhance the dissociation of H^+ and P from M and appreciably reduce the time required for the completion of the whole cycle.

The rate constants for the above given steps in the schemes (1)&(2) have been determined with a fair degree of accuracy. The calculations of basic free energies for each transition lead to the result that only 10 to 20% of free energy is available in the actual process of hydrolysis of ATP (step 3 in the first scheme and 4 in the second scheme). It is, therefore, concluded that the performance of work in muscle is associated with the steps of the dissociation of the products of hydrolysis for which the fraction of free energy released is about 60%.

The hydrolysis of ATP always occurs when the myosin heads are separate from actins since the step 3 is much faster than the time required for hydrolysis (4). It is, therefore, clear that any free energy released during the time the heads are not in contact with the thin filaments would constitute an undesirable wastage. It is nonetheless somewhat unexpected that ATP hydrolysis per se does not release free energy, or releases it by an insignificant amount, and that the major part is released during the dissociation of the products of hydrolysis. As the correctness of these findings is beyond reasonable doubt it is imperative to reanalyse the process of ATP hydrolysis and dissociation of products from the point of view of irreversible thermodynamics. What we will be interested in is not only to reascertain the well known fact that hydrolysis gives an overall amount of free energy but also the mode of its release and the role of products' dissociation.

Free Energy of ATP Hydrolysis

ATP undergoes only one reaction: it phosphorylates, *i.e.*, transfers its terminal phosphoryl group to some molecule:



$$\mathrm{AMP-O-\overset{\overset{O}{\|}}{\underset{\underset{O^-}{|}}{p}}-O\sim\overset{\overset{O}{\|}}{\underset{\underset{O^-}{|}}{P}}-O^-}\;+\;\mathrm{\underset{\underset{R}{|}}{O}-\!-H}\;\rightleftharpoons\;\mathrm{AMP-O-\overset{\overset{O}{\|}}{\underset{\underset{O^-}{|}}{P}}-O^-}\;+\;\mathrm{R\text{-}OPO_3^{2-}}\;+\mathrm{H^+}$$

In case of hydrolysis, R is simply H:

$$\mathrm{ATP+H_2O} \rightleftharpoons \mathrm{ADP+P_i^-+H^+}$$

Like for any good fuel or "energy rich compound" the equilibrium constant for this reaction has a large value (about 10^5 at pH=7). A large value of $K^{\rightharpoonup}_{e}$,

$$K^{\rightharpoonup}_{e}=\frac{k_+}{k_-}=\frac{(\mathrm{ADP})_e\,(\mathrm{Pi})_e}{(\mathrm{ATP})_e}$$

means that ATP is a strong phosphorylating agent and can drive the reaction forward against heavy odds, that is against a large concentration of products even when its own concentration takes low values. For the concentrations (ATP), (ADP), (Pi) the notations T, D and P will be used in what follows.

In a living cell, ATP concentration is greater than the equilibrium value, Te, while those of the products is much less than De and Pe. Being away from equilibrium means that the system will tend to return to equilibrium and release some amount of energy whenever the reaction is allowed to occur in the forward direction. Though such a spontaneous process is not covered by reversible thermodynamics fully, one can find out in the frame work of the latter mentioned discipline the maximum amount of work which can be extarcted from the process. This is achieved by taking recourse to a trick which is to conceive of the process to occur in such a manner that the outside forces, against which the work is to be performed, are always equal to the internal forces which result from the reaction[33]. The maximum work that the system can perform in returning to the equilibrium state is termed as the decrease in the free energy of the system (-$\triangle$G), and for a process taking place at constant temperature and external pressure the value for the maximum work, $\triangle W^{m} = \triangle G$, is given as:

$$\triangle W^{m} = - \triangle G(c/e) = - RT \ln Kc/Ke \qquad (2)$$

With Tc, Dc Pc and $H^{+}c$ as cellular conentrations of the reactants and the products (assuming water concentration to be unaffected) the above relation can be written explicitly:

$$\triangle Wm = - \triangle G(c/e) = - RT \ln \left(\frac{Dc\ Pc\ Hc^{+}}{Tc} \right) / \left(\frac{DePeHe^{+}}{Te} \right) \qquad (3)$$

This result shows that whatever be the amount of ATP in a cell, if the concentrations of products (excluding Hc^{+} at maintained pH=7)are also raised so that the ratio DcPc/Tc is made equal to Ke, ATP molecules will be totally incapable of doing any work[33]. The capacity of ATP to do work depends only on the degree to which the cellular concentrations are displaced from their equilibrium values, *i.e.*, how much is Kc less than Ke.

One cannot conceive a thermodynamically legitimate process in which an ATPase captures the free energy of ATP hydrolysis by means of a conformational change and consumes it for an associated energy requiring process and totally ignores the presence of the products in its surroundings. If it could do so then the reaction could be carried out also at equilibrium. Whatever work is performed by means of conformational change an equivalent amount of heat energy will be taken up from the surroundings since the total energy of the system must be conserved (first law). As ATP will be always regenerated from the products at the chosen equilibrium condition the following cyclical process would occur:

$$ATP + H_2O \longrightarrow ADP + Pi + H^{+} + \triangle W(\text{conformation})$$

$$\triangle Q\,(\text{heat from medium}) + ADP + Pi + H^{+} \longrightarrow ATP + H_2O$$

But this is exactly what is prohibited by the second law of thermodynamics-perpetum mobile of the second kind! Therefore, a machine of the type shown in Fig. 6 cannot exist in nature. The ATPase must be able to fele the presence of product molecules somehow. Suppose that a spring-like component exists in the ATPase, which is stretched each time ATP is split, and stores an amount of deformational energy, $((1/2)k\triangle x^2)$. While releasing this stored energy quota, the ATPase must take into account the concentration of the products around it. In a model like the crossbridge model, the quota of energy released for every step in the dissociation of the products (which are supposed to corresponds to the different attitudes of the crossbridges during the power stroke) has to be a continuous function of the concentration of Dc and Pc in sarcoplasm. The arguments in favor of [34] and against [35] such a possibilty will not be gone into here. One comment will be made however. There is no evidence of a conformational change in biology which is not abrupt; conformational changes cannot be compared to the stretching or shortening of a spring and carried out in a gradual smooth manner. It is not plausible to conceive of macromolecules as simple springs!

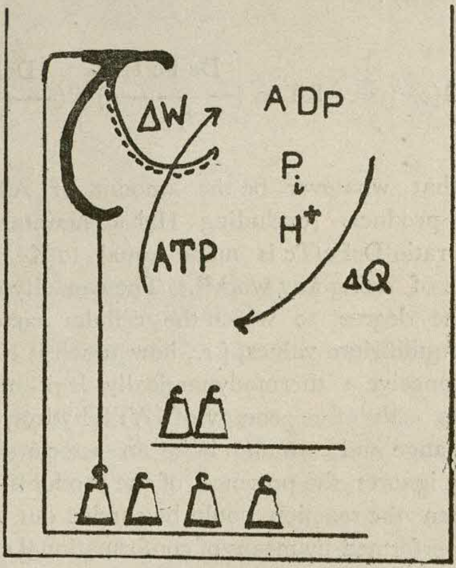


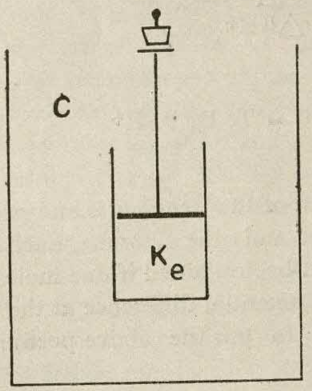
Fig. 6. An imaginary ATPase that can perform work at equilibrium eoncentrations of the reactants and products is a Maxwell's demon. Here the ATPase is shown to undergo a conformational change each time ATP is split and lift a small load.

Leaving the above mentioned problem to be resolved by those who favor the hypothesis of direct chemo-mechanical energy transduction in biology and specifically in muscular contraction, one may explore other possibilities. Though there are no cylinders and pistons involved in the hydrolysis of ATP in living cells a lot can be learnt from reactions in which gases are generated and perform work during their expansion. In the combustion chamber (Fig.7), the fuel burns and generates a high pressure of gases upto the limit when equilibrium is achieved between the partial pressures of the products and the reactants. If the pressures of the product gases are less in the surrounding medium the gases expand and deliver some work output. The maximum work output

$$\triangle W_m = -\triangle G = -RT \ln K/K_e, \tag{4}$$

where the constants K and Ke are the ratios of the product of partial pressurses of the product gases and the reactants in the surroundings and for the situation of equilibrium. Now in the case of a combustion process in a chamber we do have an identifiable region where Ke is achieved. This information is missing in the case of ATP hydrolysis in living cells. Eq. 2 gives us the value of maximum work output for a nonequilibrium Kc value in the cytoplasm but there has to be some region in the cell where the high values of the concentrations of the products are obtained to produce the equilibrium ratios, Ke. Since ATP is hydrolysed within a protein complex, the ATPase, it is natural to identify ATPases as the reaction chambers in which ATP hydrolysis generates an equilibrium ratio of the concentrations of products and reactants. This may be formally stated as a hypothesis :

Hypothesis 1. ATPases are reaction chambers for ATP hydrolysis in which the reaction of hydrolysis achieves, or tends to achieve,equilibrium concentrations of products and reactants.



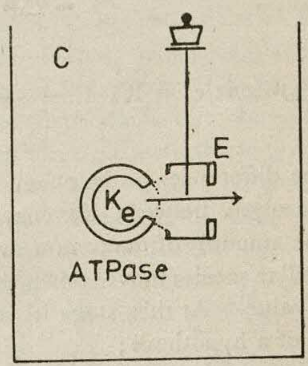


Fig. 7. A thermodynamic system comprising a cylinder and the surroundings, here a container, is shown. Combustion of a fuel in the cylinder causes the build up of high partial pressures of the product gases within the cylinder upto the equilibrium value (Ke). Work is performed when the product gases expand against the external pressure and the weight which is put on the piston.

Fig. 8. An ATPase is depicted as having a small capacitance for the product(s) of ATP hydrolysis, within which equilibrium value (Ke) for the reaction is achieved. This leads to the generation of high chemical potential(s) of the product(s) in the ATPase. Work can be performed by a yet unidentified engine, E, which is capable to utilize the energy released in the downhill movement of the product(s) from the ATPase to the cytoplasm, C, so long $K_c < K_e$.

In Fig. 8 an ATPase is shown with a small capacitor in which the products of hydrolysis are trapped. The surrounding medium is cytosol containing low concentrations of the products and high level of ATP. For this system the free energy change per mole of ATP hydrolysed is given by the following equation where "enz"=ATPase,

$$\begin{aligned}\triangle G &= RT \ln Kc/Ke = RT \ln Kc\,Kenz/Ke\,Kenz \\ &= RT \ln Kc/Kenz + RT \ln Kenz/Ke \\ &= \triangle G(\text{cytoplasm/enzyme}) + \triangle G(\text{enzyme/equilibrium}) \qquad (5)\end{aligned}$$

If we let

$$Kenz = \frac{Denz\ Penz\ H^{+}enz}{Tenz} \longrightarrow Ke = \frac{DePeH^{+}e}{Te} \qquad (6)$$

then $\triangle G(\text{enzyme/equilibrium})$ will tend to zero and we get
$\triangle G = \triangle G(\text{cytoplasm/enzyme})$.

One can transform the expression for $\triangle G(\text{cytoplasm/enzyme})$ into a very useful form as follows :

$$\begin{aligned}\triangle G(\text{cytoplasm/enzyme}) &= RT\ \mathrm{In}\ (Tenz/Tc)(Dc/Denz)(Pc/Penz)(H^{+}c/H^{+}enz) \\ &= \triangle\mu ATP(enz/c) - \triangle\mu ADP(enz/c) \\ &= \triangle\mu Pi(enz/c) - \triangle\mu H^{+}(enz/c) \qquad (7)\end{aligned}$$

Here

$$\triangle\mu i(enz/c) = RT\ \mathrm{In} \frac{(i)\ enz}{(i)c},\ (i) = T, D, P, H^{+} \qquad (8)$$

are the differences in the chemical potentials of the reactants and products of hydrolysis between the enzymic chamber and the cytosol. Each stands for the amount of maximum work that can be obtained if one mole of the molecular species move down this chemical potential difference at the given, fixed value. At this stage it is appropriate to put the above possibility in terms of a hypothesis ;

Hypothesis 2. The release of the free energy of ATP hydrolysis consists in the release of energies of reactants and products of hydrolysis as these move down their respective chemical potential differences between the reaction chamber and cytoplasm.

In the case of a chemical reaction of a fuel producing some gases, the total pressure developed in the cylinder serves to push a piston. The total pressure is the sum of the partial pressures of the product gases and each of these contribute to the work output using the the same piston to release the energy stored in them. In the present case we are not sure whether all of the products will have a suitable machine at their disposal within the cell. Assuming only one of the products can be made to perform work when it moves down its chemical potential difference by a machine present in the cell but has not been yet indentified, how could one prevent a wastage of the free energy of hydrolysis of ATP. For this question to be answered one should see whether it is permitted by thermodynamics to enhance the concentration of the "useful" product and to reduce it for those products which would be wasted because of the lack of an appropriate machine in the cell. Now Ke contains in its numerator the product $D_e P_e H^+_e$. As was pointed out in the beginning of this section, the high phosphorylating power of ATP consists in its capacity to make this product very large even when it is present in small concentrations, with the lowest possible value given by Te. Supposing we were to artificially introduce one of the products, say Px, in the reaction chamber and then hydrolyse ATP. As all products are now generated simultaneously, the level of Px will rise with the others but always ahead of them by the initial amount. In this case the equilibrium will be achieved when the values of the concentrations of the products are different but the product of these values is again K_e x T_e. Since Px_e is greater than the rest its chemical potential difference will be also greater than those of the coproducts. If we now release one molecule each of ADP, Pi and H^+, Px flux from ATPase to cytoplasm will release more energy than the others. In other word ATP hydrolysis at this imbalanced concentrations, ratios of products will lead to the release of the major part of the energy of hydrolysis via the channel of Px. Thus if living cells have only one machine at their disposal which can harness the flux of only one of the products of ATP hydrolysis the ATPase can increase the efficiency of the utilization of the free energy of hydrolysis by selectively raising the concentration of one of the products at an initial stage by hydrolysing ATP and releasing all other products but the selected one. Working thus the ATPase will be in a position to divert the major part of the free energy of hydrolysis through the chemical potential difference of the "useful" product. In the extreme case the ATPase could achieve the limit: Kenz = Ke; keep the concentrations of the non-useful products, (n–u, 1) and (n–u, 2) in the capacitor the same as these are in the cytoplasm; keep Tenz = Tc and take the concentration of Px, the useful product, (u)enz to the uppermost limit:

$$(u)enz = Tc\ Ke/(n-u,1)c \times (n-u,2)c.$$

In this ideal situation,

$$\triangle G = -RT \ln [(u)enz/(u)c] = -\triangle\mu_{Px}\ (enz/c)$$

and all of the free energy of ATP hydrolysis will be available as the chemical potential difference of the useful product. It should be noted, however, that this will be true only if the ATPase does not discharge all of its stored stock of the useful porduct, Px, in one go, but to release one product molecule for every molecule of ATP hydrolysed. If it does release more than one Px molecule to the cytoplasm through the unknown machine it will have to charge itself up again to attain full efficiency.

We will now give some reasons which point to H^+ as the useful product, Px.

1. In the case of the reversible ATPase of mitochondria and H^+-ATPases associated with cell membranes[33],[36] ATP hydrolysis is known to produce an electrochemical potential difference of H^+ on the two sides of the membranes. Here the outer surface of the membrane serves as the capacitor for storing H^+ along with the whole extracellular space.

2. In the very precisely studied case of myosin ATPase it has been found that whereas ADP and the phosphate ion, Pi, are released into the solution during the early rapid hydrolysis of ATP, proton early burst has a substantially smaller magnitude[37]. It is, therefore, not possible for ADP and Pi to accumulate in the ATPase and have a high amount of energy release associated with their fluxes. Accumulation of ADP is not possible anyway since its occupation of the hydrolytic site would block ATP getting bound to the same site. Unlike for Pi which is a massive ion H^+ can be easily accommodated at NH_2 groups in the hydrolytic site and other groups which can be protonated both within the hydrolytic site or elsewhere in the enzyme.

The main reason for identifying H^+ as the useful moiety among the products of ATP hydrolysis is that there is a particular mechanism conceive by which the release of the energy during its downhill movement from the ATPase to the cytoplasm can be harnessed for mechanical work. This will be the theme of the next section. For the moment we will summarize the thoughts expressed above in terms of a hypothesis :

Hypothesis 3. In order to work efficiently an ATPase should preferentially accumulate protons to the maximum value permitted by the equilibrium constant K_e and release them in small numbers whenever required for work output coupled to their migration down their maintained high chemical potential difference.

Before we proceed to discuss the proton driven motor in the sarcomeres a sequel of the third hypothesis will be mentioned. In order to have a continued high power output under the constraints of Hypothesis 3, there is a need of a large number of ATPases working alternately. Since each of them will have to charge itself up to the equilibrium concentration, from a level which is kept close to the equilibrium value (large μH^+ (enz/c) the process of recharging will be much slower than during an intial phase of ATP hydrolysis.

Though we have been able to narrow down the choices of how the free energy of hodrolysis of ATP is used within the cell the final answer to the problem is yet to be given. Following the analogy of combustion of a "good" fuel (K_e large) and the mode of release of energy in reactions producing products in gaseous state, it has been suggested (*Hypothesis 1.*) that ATPases are a kind of reaction chambers (cylinders) in which a high chemical potential of the products (limited by Ke and Tc) can build up. The same analogy led to the idea of release of energy of the reaction of hydrolysis by the downhill movement of the products from the AT Pase to the cytoplasmic medium where their chemical potential is low (*Hypothesis* 2.). Requirement of high efficiency in the utilization of $\triangle$ G ATP forces one to choose H^+ as the "useful" product whose chemical potential in the enzyme is kept maximal (*Hypothesis 3.*) and which is released in small quota by a large number of ATPases during periods of sustained high power output. These results follow from rigorous application of the laws of thermodynamics. There are two deficiencies which remain to be removed: 1. why should H^+ be selected by nature as the useful product? and 2. how to get vectorial effects (flow or force) from the chemical reaction? The answer to both of these pending questions comes from the analysis of actomyosin systems (including the sarcomere) in the light of Curie–Prigogine Principle[38] with the extension of the principle by Katchalsky[39]. This principle states that, unless a medium in which a reaction takes place is anisotropic, no vectorial effect can ensue from a chemical reaction.

In an isotropic medium a chemical reaction consists in the removal of a reactant at the same rate in every part of the system and, similarly, the production of new molecules also at the same rate in every part. There is no imbalance in the concentration of any participant in the reaction in the solution. Therefore, there is no chemical potential difference generated by the reaction which would lead to a directional flow of mass or energy (vectorial effects). Thus in an isotropic medium the principle prohibits the occurence of vectorial effects arising from chemical reactions. That this is permitted to occur in an anisotropic medium may be exemplified by a chemical cell which can cause a flow of electrons from one electrode to another so long the electrode materials are different, *i.e.*, so long anisotropicity exists in the system.

Let us have a look at the overlap region in a sarcomere. As far as the distribution of S1 (heads containing the ATPase) is concerned the medium is isotropic. Thus the reaction of hydrolysis, upto the moment of dissociation of products, is ruled out as a cause for muscle contraction (generation of a longitudinal force). Next comes the stage of the delivery of reaction products from the AT Pase which occurs during the interaction of the heads with the actins of the thin filaments. Again if the products are released in the interfilamental spaces there cannot be any vectorial effect generated in the process, because their release would be, on an average, to the same extent in every small subregion of the overlap zone. Thus the only way to get vectorial effects is to release the products, or at least one product, in the filaments themselves and to consider the myofilaments as having an inbuilt anisotropicity as regards the flux of the product (s) in them. Now it is inconceivable that ADP and Pi could travel within the myofiloments to regions outside of the overlap zone. On the other hand it is possible for H^+ to do so. In the ∞ –helical state proteins are maximally hydrogen bonded and form proton wires[40–42].

The process of H^+ conduction will be discussed in some detail in the next section. At the moment we hold on just to the fact that only H^+ has the faculty of moving within the filaments. However, this too is insufficient for getting longitudenal anisotropicity unless this movement is possible in only one direction within the filaments.

The most simple actomyosin system displaying vactorial movement is that of F–actin and dispersed myosin molecules. F–actin is seen to move in one direction upon ATP hydrolysis like thin long submarines[43]. If F–actin bundles are fixed to a surface then one sees active streaming when ATP is split by myosin or HMM or S1[44]. The anisotropic nature of the F–actin is evidenced by the different rates of its polymerisation at its two ends and in this particular case no myosin AT Pase is involved[43]. What are the implications of these observations for the proton conducting system of F–actin ? Is there any plausible reason to believe that proton conduction in proteins and protein complexes can be unidirectional ?

Proteins have, apart from their inherent assymmetry as polypeptides in having an NH_2– and an –COO^- terminal, also very varied spectrum of weak and strong acidic and basic groups. At neutral pH all COO^- groups are dissociated, reflecting a lack of willingness on the part of COO^- to share its electron with H^+. The amino groups can be in either of the two states : NH_2 or NH_3^+. Now if a T–shaped proton wire is considered, as shown in Fig. 9, having, apart from the N–and C–termini, a side chain carrying a basic group, one can perform the following thought experiment :

1. The C–terminal is offered an H^+ and the N–terminal an OH^-.
   Result : COO^- refuses to accept the proton ; there is no H^+ transfer across the proton wire,
2. The N–terminal is offered an H^+ and the NH_3^+ group of the side chain an OH^-,
   Result : H^+ move from N–terminal to protonate the NH_2 group of the side chain (which was neutralized by OH^-)
3. The NH_2 group of the side chain is offered an H^+ and two OH^- to the COO^- and NH_3^+,
   Result : H^+ move from the side chain to the N–terminal but not to the C–terminal.

All in all the C–terminal is a dead end of the proton conductor since it is not prepared to accept a proton from any quarter. The result of experiment No. 3, deduced from the titration behavior of the ionizable groups of proteins, gives us the possibility of assuming an inherent assymmetry of proteins. These deliberations can be formulated as a hypothesis :

Hypothesis 4. An ATPase can generate a vectorial flow or force only in conjunction with a proton, conducting protein (protein assembly) by injecting protons, produced in ATP hydrolysis, at the NH_2 group (s) of the side chains.

It may be noted that the assymmetry lies basically in the possibility of a

H^+ neutralizing reaction only at one of the two terminals of the proton wire, and not in the proton wire itself. If the N–terminal is blocked by the addition of some group (e.g. by DNFB) it would not be possible to cause any proton flux in the wire in any direction. Furthermore, if the pH at the N–terminal is made sufficiently acidic, the chemical potential of H^+ at the exit point will become the same as in the H^+–capacitor of the ATPase and again no H^+ flux would occur.

So far we have let us be guided by thermodynamics and reached certain conclusions about the nature and mode of release of the free energy of ATP hydrolysis. Hypothesis 4. has extended the results of Hypotheses 1–3 to the case in which one is intereseted in obtaining a (global) vectorial effect from the hydrolysis of ATP. In the next section, the basic principles of electrochemistry will be used to obtain "the basic biodynamic phenomenon". Though this will be substantiated in the next section, for the sake of completeness of this section we will formulate the result of the things to follow as our final hypothesis :

Hypothesis 5. When a large number of ATPases, which have generated a high chemical potential of H^+ within them, inject H^+ in a macromolecular assembly of proteins with H^+ neutralization capacity at one side of their proton conducting system, a flux of H^+ occurs from the sites of injection of H^+ to the sites of their neutralization. This H^+ flux is charge compensated by a flux of counterions around the macromolecular assembly. The electroosmotic movement of the counterions along with the solvent (water) causes an active streaming to occur along the protic cable which can be harnessed by living cells for all biodynamic purposes.

The Basic Biodynamic Phenomenon

Once a scientist is relying on the established basic principles of the exact sciences it is not impertinent to call the results which are obtained in this manner as basic or fundamental. Our *tour de force* of the previous section has been hard enough and some amount of hard excercise is still left to achieve the goal. At the same time one can be confident of the results and carry the necessary conviction of a scientist.

The main question which is to be answered in this section is the consequence of proton flux in one direction along a macromolecular assembly of proteins in a cell. To treat this question appropriately one should know the concepts from the field of electrochemistry which pertain to protonic couductance and interfacial phenomena. These are not difficult to explain or to understand, at least qualitatively. First the matter of proton conduction will be taken up followed by the subject of the phenomena of electrical double layers and electrokinetics.

Although ice has neither free electrons in the inter-lattice spaces nor freely mobile anions and cations like in a solution of dissolved salts, it has a fairly high electric conductance, called the anamolous conductance of ice[45]. This effect has been explained in terms of the Grotthus–Onsager theory[45],[46]. The basic idea of the theory is that ice forms a structure with a continuous system of hydrogen bonds and that the charge transfer occurs by means of a propagated protonation of water molecules followed by a rotation of water molecules to their initial state. Onsager and others[40],[41] have extended this theory to proteins. Proteins have in the ∝-helical form maximum hydrogen bonding between the H atom attached to the electronegative N atom of one peptide linkage and the oxygen of the carbonyl group of the fourth amino acid. So long the secondary structure of a protein is undisturbed it is a perfect proton wire; there is a minimal activation energy barrier for sideways shift of H^+. On the other hand, local or global breaking of the natural hydrogen bonded complex can abolish their proton conducting faculty.

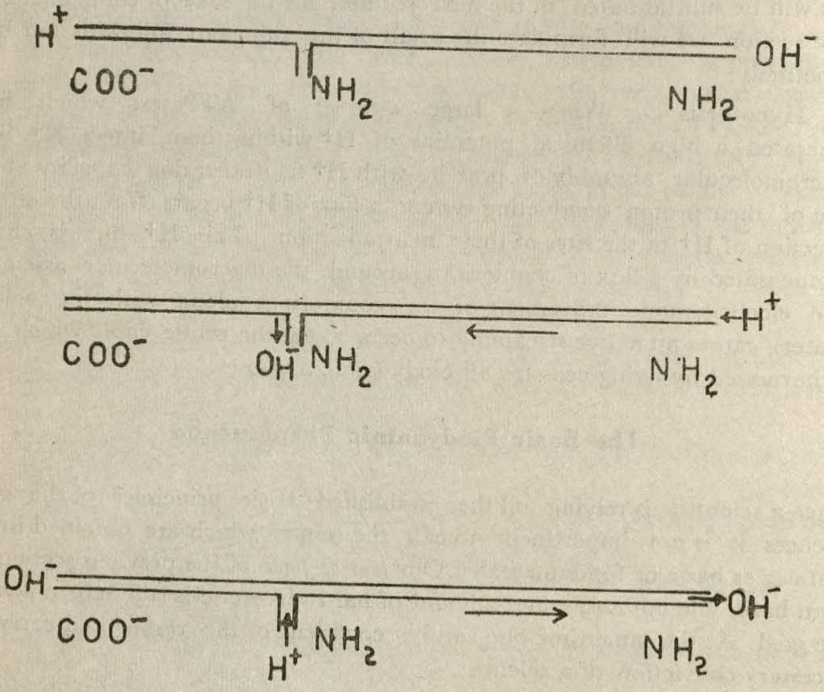


Fig. 9. Occurrence of vectorial flux of H^+. See text for explanation.

Suppose that a filamentous macromolecular assembly is formed by organized aggregation of proteins and the resulting filament bears an excess of carboxylic groups over amino groups towards the aqueous phase. Further it is assumed that the filament has as a whole proton conduction property, has an overall N–C polarity and has NH_2 groups located at specific sites over its surface. Under these assumption we will now show that a peculiar electroosmosis will occur along the surface of the filament when an ATPase injects H^+ in the side chains bearing NH_2 groups.

The theory of electric double layers[45] tells us that the filament will be surrounded by a cationic layer in a narrow region of the aqueous phase, Fig. 10, while the anions will be displaced away from the net negatively charged surface of the filament. In living cells potassium ions are the major cationic species and we will call the diffuse cationic layer simply the K–layer. From the phenomenon of electroosmosis[45] it is known that the diffuse cationic layers have a high surface conductance, that is, the layer moves readily under the influence of an electric field applied tangentially to the surface. It is also known that this lateral movement of the cationic layers causes a movement of water by means of a viscous coupling between the cations and water molecules at the surface (hence the name: electroosmosis). In the case of our filament the theory predicts a lateral movement of K^+ along with water whenever an axial electric field is applied along the length of the filament.

When some dispersed ATPases in the medium around the filament inject H^+ in the side chains (via NH_2 groups) H^+ will start coming out at the N–terminal(s) of the filament. This will create an imbalance in the electric charge density between the N–terminal and the region of injection of H^+. Neither of the co-products, ADP and Pi, have a high mobility and it is out of the question that these could quickly move over to the N–terminal of the filament and bring about an equalization of electric charge. The density of Cl^- is about 1/20th of K^+ in cells and worse even, the Cl^- are kept far away from the surface where the lateral component of the electric field is maximal (Fig. 10). Thus the major effect of the electric field will be to cause the K–layer to move in the opposite direction to the movement of protons.

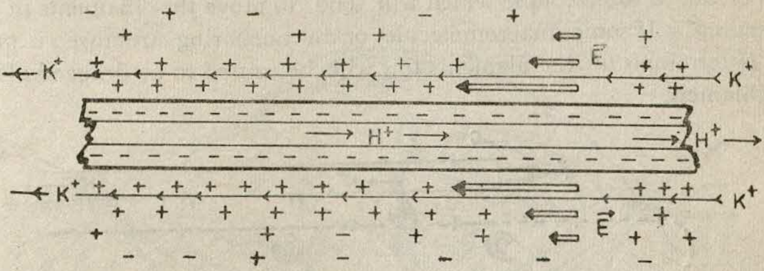


Fig. 10. A filamentous assembly of proteins is represented schematically. The backbone of the filament has proton conducting system of hydrogen bonded chains, while the surface bears negative fixed charges. There is a high density of cations (K^+) surrounding the filament on all sides, as a cylindrical shell. The electric field generated by H^+ flux under the action of a chemical potential difference across the length of the filament, acts fully on the cations, causing an electroosmotic flux of the laterally mobile K-layer.

This phenomenon of H^+–flux–mediated electroosmosis was initially deduced in plants for the case of H^+ migration along membranes which form the lining of the transport system in plants. There it was assumed that proton transport occur in the structured layers of water at the surface of membranes. The quantitative treatment of H^+ flux mediated electroosmosis

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or protoosmosis has been given elsewhere both for the case of filaments and tubular systems and will not be gone into here. In this section some qualitative features of the effect will be dicussed with their possible applications in biology.

As the electroosmotic flow occurs along the filament two effects can arise from it:

1. If the filament is free to move in the medium it will be propelled in the direction of the C terminal(s) by the viscous force of the moving K–layer. Here again no out side electric field has been imposed in the medium, the field is generated by H+ migration itself. This is a vectorial effect caused by an ATPase in conjunction with a proton wire with an inbuilt asymmetry.

2. If the filament is fixed in its position only an active streaming will occur at its surface. The direction of the active streaming will be again towards the C–terminal.

It is suggested that in living systems the first effect is responsible for the various forms of contractility. Muscle will be discussed in the next section in detail, here it will suffice to give the essentials of a contractile unit, (Fig.11), and apply it to the movement of chromatids towards centriols during mitosis. In the bsaic contractile unit two filaments have been laid parallel to each other with reversed directions of their N– and C– terminals. An ATPase which can interact with the side-chain NH_2 groups of the filaments (actin filaments or microtubles) is present in the medium. Under physiological conditions the ATPases contact the filaments and deliver H+ into the filaments. The K-layer will move along the two filaments in opposite directions and create a force couple which will tend to move the filaments in opposite directions. If some macromolecule or an anchoring structure is present at the N-terminals of the filaments this will be caused to be dragged along with the filament.

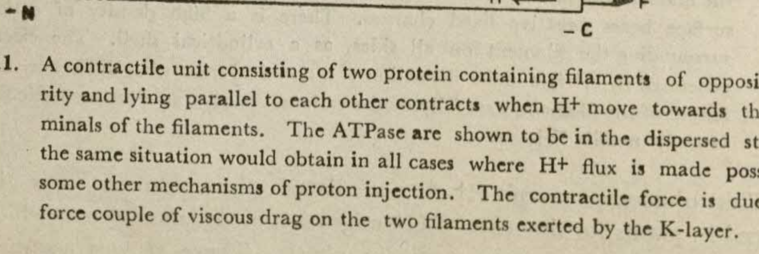

Fig. 11. A contractile unit consisting of two protein containing filaments of opposite polarity and lying parallel to each other contracts when H+ move towards the N-terminals of the filaments. The ATPase are shown to be in the dispersed state, but the same situation would obtain in all cases where H+ flux is made possible by some other mechanisms of proton injection. The contractile force is due to the force couple of viscous drag on the two filaments exerted by the K-layer.

In mitosis (anaphase) the situation is slightly more complex than the working of the above given single contractile unit, but lends itself amenable to a fairly clear treatment within the framework of the protoosmotic phenomenon. From our point of view the relevant informations are as follows :[46]

(1) The polar microtubules have their (–) terminals (COO^- groups) towards the equator, while the positive ends are buried in the polar complex.

(2) The kinetochore microtubules have their (+) ends buried in the kinetochore and the (–) terminals are free and directed towards the ploes.

(3) GTPis hydrolysed during active polymerization at the growing (–) terminals of the polar microtubules.

The hydrolysis of GTP at the terminal points of the polar microtubules will inject H^+ there and these will move out from the NH_3^+ groups probably located in the polar complex (centriole along with the dense material around them). The result will be the pattern of active streaming shown in (Fig. 12). This will have the following consequences. a) The mitotic spindle will be stabilized in the fluid medium for its functional role, and provide a net outward force (with respect to the equator) acting on the two poles. In the region of overlap between the polar microtubules there will be a contractile force which will balance the out ward force in the early anaphase and serve to maintain the fluid movement in the central region. b) Apart from a possible minor movement of H^+ in the kinetochore microtubules towards the

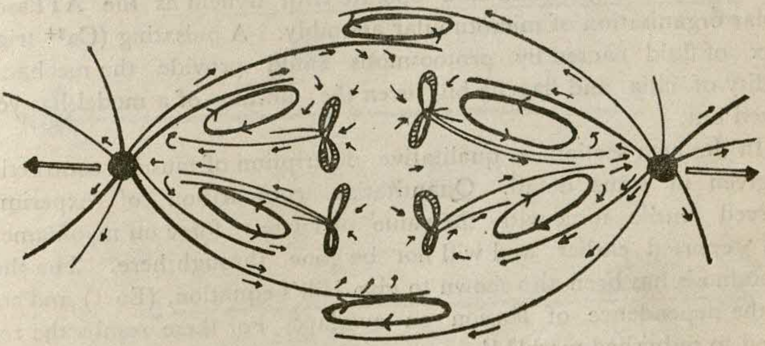

Fig. 12. The dynamics of the mitotic spindle is shown to result from electroosmotic flows along the microtubules. Proton fluxes are towards the centriols (black circles) and K-layer movements are towards the growing ends of the polar microtubules where GTP is hydrolysed. The outward forces on the poles arise from the radially outward electroosmotic flows away from the equator. In the region of overlap between the polar tubules there is a centrally directed contractile force, partially balancing the outwards forces. Chromatids are pulled towards the poles by the viscous drag of the fluid movement along the kinetochore microtubules.

chromatids (in case some not yet discovered dynein-like ATPase is involved) the kinetochore microtubule will be thought to be inert structures since these do not show any polymerization during anaphase. These microtubules are pulled towards the poles by the viscous force of the hydrodynamic streaming, specially close to the poles. In the latter stages of the anaphase the terminals of the kinetochore microtubules depolymerize as the chromatids approach the poles.

The action of the contractile ring formed by F-actin is very close to our basic model of contractile unit. Naturally in this process it is the myosin ATPase which serves to cause protoosmosis. It may be noted that there is no evidence of the presence of thick filaments in the contractile ring[46]. Similarly there is no evidence of dynein associated with microtubules in the mitotic spindle. In both cases the crossbridge model or its extension to microtubules having dynein are not applicable. At present there is no known mechanism by which cell division dynamics could be explained. Protoosmosis seems to be appropriate as the dynamic principle for this process though the details of the above given approach-which is merely a model, may be incorrect.

Regarding the applications of protoosmosis for the second case (fixed filaments) one may mention cytoplasmic streaming[47] and transport over long distances within individual cells, such as axons[48] or in tissues, such as phloem[49,50]. Cytoplasmic streaming occurs via actomyosin interaction while microtubules are responsible for long distance transport in axons. The basic principle of protoosmosis can be fruitfully applied in both cases. Transport in plants has been worked out in some detail by the author elsewhere[50].

Motility in flagella and cilia operate with dynein as the ATPase and a copolar organisation of microtubular assembly. A pulsating (Ca^{2+} triggered) influx of fluid caused by protoosmosis could provide the mechanism for motility of cilia and flagella but even the outline of a model has yet to be worked out.

In the next section a qualitative description of muscle contraction will be given in some detail. Quantitative comparision of experimentally observed tensile force with the value of viscous force on myofilaments has been reported earlier and will not be gone through here. The theory of protoosmosis has been also shown to give Hill's equation, (Eq.1) and correctly give the dependence of tension on overlap. For these results the reader is refered to published work[12-14].

The Mechanism of Muscle Contraction

As against the discource of the mode of liberation and utilization of free energy of ATP hydrolysis and the theory of protoosmosis which were dictated by the laws of thermodynamics and obtained from the basic principles of electrochemistry, the model of muscle contraction given below does not have any inviolable foundations and could be found wrong or deficients in many a details. Nonetheless the model gives the right quantitative results in terms of orders of magnitude and resolves several outstanding problems in a simple and consistent manner. However the reader should feel himself free to reject some of the assumptions made here by the author to obtain a consistent description of contraction process.

The contractile unit of the sarcomere is conceived to operate in the following manner.

In a relaxed muscle SI are loaded with protons which result from the hydrolysis of ATP during the recovery phase. The proton wire of the thick filament is in working condition but some hydrogen bonds are broken in either S2 or at the junction of S2 with S1. Thus H^+ cannot leave S1 along the thick filaments and reach the M-line where CPK or buffers could neutralize these.

The thin filaments have their negative terminals towards the H-band, and the only way for H^+ in these filaments is towards the Z-disc where these could be consumed either by the Lohmann's reaction (CPK is present both in the M-line and the Z-disc)[51] or neurtalized by buffers. However, in the relaxed muscle the proton cable of the thin filaments is assumed to be disconnected at the sites occupied by the troponin assembly. Thus even if the myosin heads were to contact the thin filaments these would merely convert all NH_2 groups of the side chains of G-actins into NH^+_3 but no flux of H^+ would occur upto the Z-disc. The situation in the relaxed muscle is presented in Fig. 13.

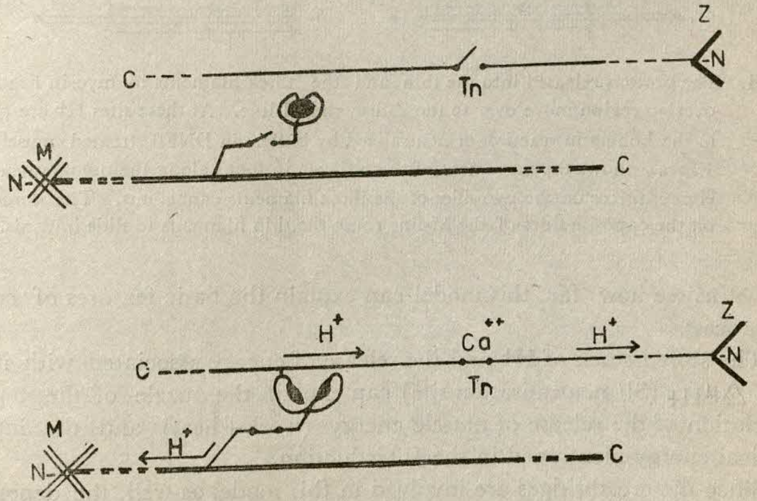

Fig. 13. The regulation of the activity of actomyosin ATPase in muscle is conceived to be associated with the switching on and off of the proton circuits of the myofilaments. In the particular case of Troponin mediated control, shown in the diagram, Ca^{2+} are assumed to re-establish (lower diagram) hydrogen bonded chains in the thin filaments which are broken by the interaction of Tn-I with the G actins in a relaxed muscle (upper diagram). The interaction of one S1 with actins is thought to simultaneously establish the proton circuit of the second head with the thick filament.

The role of Ca^{2+} is seen to switch on the proton wire of the thin filaments by re-establishing (through the mediation of the Tn subunits) the hydrogen bonds between the pairs of actins covered by Tn. This enables H^+ of the NH_3^+ groups of the actins to migrate to the Z-disc. This also alters the charge state of the thin filaments and enables the myosin heads to contact the G-actins. At the moment of contact of one S1 with G-actins the hydrogen bonds in S2 or S1/S2 juction are re-formed so that the protons can move over from the two heads both to the thin as well as to the thick filaments as shown in Fig. 13. The contractile machine can now start to operate as described below.

The proton flux along the thin filaments is towards the Z-disc and that along the thick filaments towards the M-line. The K-layer moves in the opposite directions along the filaments to give rise to a hydrodynamic flow in three dimensional loops. These flow patterns and the resulting force couple due to the viscous force exerted by the flow on the filaments are shown in Fig. 14. Contraction stops by the withdrawl of Ca^{2+} by SR after the cessation of neuronal pulses.

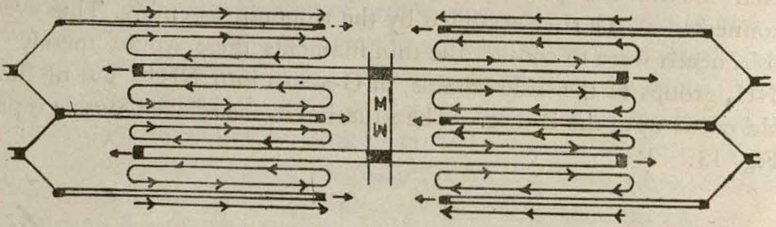

Fig. 14. The protons released into the thin and the thick filaments by myosin heads in the overlap region move over to the Z-disc and M-line. At these sites H^+ are consumed in the Lohmann reaction or neutralized by buffers in DNFB treated muscles. The diagram shows the movement of the K-layer in loops along the myofilaments. The viscous forces on the two sides of the thick filaments cancel out. The force couples on the opposite sides of the M-line cause the thin filaments to slide inwards.

Let us see how far this model can explain the basic features of contraction process.

The stored stock of H^+ and the release of energy associated with its flux down $\Delta\mu_{H+}$ (Sl/ neutralisation site) can explain the puzzle of the temporal dissociation of the release of muscle energy (work+heat) and the input of chemical energy mentioned in the Introduction.

Since the crossbridges are involved in this model as well, the dependence of the tension on the degree of overlap remains the same as for the crossbridge model as regards the input of chemical energy. However, the crossbridges serve mainly as capacitors of H^+ which serve to pass on H^+ to the proton wires of the thick and the thin filaments. These do not participate in the process of the generation of mechanical force. The viscous force couple is active in the region of overlap alone and this provides for the correct dependence of tension on overlap as regards the hydrodynamic (mechanical) coupling between the two sets of filaments.

In order to get the Fenn effect we note that the rate of the injection of H^+ on the thin filaments will depend on the number of non-protonated NH_2 groups. This will depend on the rate of H^+ crossing the I-band i.e., on the net flux of protons from the region of the overlap to the Z-disc. This flux of H^+ depends on the degree to which the electric field, generated by their own flux initially, is unbuilt by the charge compensating movement of the K-layer. As there is a viscous coupling between the lateral movements of the K-layer and the thin filaments the K-layer moves with less hinderance if the thin filaments move in the same direction as the K-layer. This situation obtains during shortening. Therefore, during shortening there will be a greater output of muscle energy than in isometric contraction because H^+ flux will be greater.

The very curious situation observed during stretching of muscle[52] which cannot be explained by the crossbridge model, *viz*, the generation of greater tension than T_o at about 1/20 th consumption of ATP (for the same duration of contraction) is explained as follows. Since the viscous force is proportional to the relative velocity of the filaments and the K-layer, the tension generated during the stretching of muscle must be greater than T_0. At the same time the charge compensating flux of the K-layer is hindered by a counterdirectional movement of the thin filaments and, therefore, the flux of H^+ would be reduced. From the reasoning given in the above paragraph it follows that the rate of ATP hydrolysis will fall in the case of stretching of muscle.

Discussion

The contents of this article can be classified in three categories according to the degree of reliability which can be associated with them. Two items, *viz.* the new concept of the nature of free energy of ATP hydrolysis and its conversion, via protoosmosis, have their foundations in the inviolable principles of thermodynamics and electrochemistry. Thus the part concerning the fundamental aspects of the "basic biodynamic phenomenon" can be considered to have a high degree of reliability. The matters relating to the role of protons find full support from the well established chemiosmotic theory of Peter Mitchell[53]. Protoosmosis provides a unification of bioenergetics of energy transducing membranes with the bioenergetics of motility and contractility. The crossbridge model has been a puzzle for the adherents of chemiosmotic theory but, because of the absence of a known mode of energy transduction from $\triangle \mu H^+$ to a mechanical form the unification of the biodynamic process of active transport with motility and contractility could not be achieved. Protoosmosis provides the link.

The second aspect of this paper relates to quantitative considerations of the relevance of protoosmosis for muscular contraction. Though not covered in this article explicitly in order to avoid repetition, it is important to emphasize that the viscous force on the myofilaments exerted by the K–layer is of sufficient magnitude to account for the tension in muscle. This part of the theory of muscle contraction does not depend on certain assumption of purely conjectural nature on the mode of action of Ca^{++}. Thus in the one case where sufficient experimental data is available on a biodynamic process the protoosmotic theory gives a correct quantitative description of the phenomenon.

For the past thirty years lot of effort has been put to verify the crossbridge model and to understand contractility and motility on the basis of this model. There is lot of information available on such aspects of these phenomena which relate to conformational changes (basically the lack thereof), structural details of the assemblies of such units, etc. Many details required for elucidating the dynamic phenomena from the perspective of protoosmosis are not available. The author is, therefore, compelled to make certain assumptions which have the least degree of credibility. Among these assumptions are the proton conduction properties of the myofilaments and their subunits. From the susceptibility of S2 to attack by proteolytic enzymes it would be plausible to assume that this subfragment of HMM may have a discontinuity in its hydrogen bonded structure at these points but not much is known about S2 properties in this respect. Similarly the steric blocking model of the regulatory proteins is tailor made for the crossbridge model. Though it is known that the crossbridges do not adopt the rigor attitude during contraction the concept of precise steric interaction between the crossbridges and the actins continues to dominate the thinking of the research workers in this area. The proposal of discontinuity in the proton wire in the thin filaments is a pure conjecture. Thus the proposed events accompanying the activation of the contractile machinary are of the least reliability.

Though some conjectures have been made about mitosis more information is required to understand the GTP hydrolysis activity of tubulins, the kind of cappings that are involved at the C-and N-terminals of microtubules in the poles and chromatids and the side chains of tubulin. Inspite of its tentative character the model is the only one existing to date with some degree of plausiblity.

To conclude, there are a number of questions which have to be clarified to come to a full fledged theory of muscle contraction and motility and contractility in general. In protoosmosis one has the foundation of a new edifice. The principle is versatile enough to cover all of the biodynamic phenomena with the diverse organizational patterns formed by living cells for specific purposes. It has solid foundations in exact sciences and forms a natural link with the allied field of active transport and energy transduction in the membranes of mitochondria and chloroplasts. It is hoped that future scientific investigations will be directed to provide informations required to put the models based on protoosmosis on firm theoretical and experimental foundations.

Acknowledgements

The author wishes to express his gratitude to Sir Andrew Huxley and Professor Hugh Huxley for the helpful discussions he had with them about the present model of muscular contraction. The author also wishes to thank Professor R.C. Woledge for his advice and guidance on future experiments for substantiating protoosmosis.

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2

REGULATION OF EUKARYOTIC PROTEIN SYNTHESIS

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Introduction

The molecular constituents that most directly determine the struture and function of cells are proteins. The information for the synthesis of proteins is stored in DNA which is expressed through two distinct steps called transcription and translation. The transcription is the first step in which genetic message in DNA is copied into RNA, whereas in the second step the message is decoded into protein on the ribosomes using messenger RNA (mRNA) as a template. In a cell, relative amounts of the different proteins vary enormously depending on their function. Moreover, different tissues of an organism synthesize different kinds of proteins inspite of the fact that all tissues of an organism have same genetic composition. All this, obviously, means that there is differential synthesis of proteins indicating the existence of molecular devices that regulate differential expresstion. There are at least two ways by which cell can achieve the differential synthesis. Firstly, by developing molecular signals that control the rate at which specific mRNA molecules are transcribed (transcriptional control). Secondly, by developing molecular devices to control the rate at which mRNA molecules are translated into polypeptides (translational control). An understanding of control mechanism (s) of transcription and translation would provide us answers to many puzzling questions related to development and differentiation (*i.e.* expression of the right genes in the right cells at the tight time), cancer biology (over/misexpression of oncogens) and immunology (expression of immunoglobin genes).

Number of investigators have concentrated for the last three decades to unravel the mechanisms that control transcription. Genetic and biochemical studies in prokaryotes have yielded a lot of important information, and a set of principles for the regulation of transcription. However, only recently it is equally appreciated by molecular biologists that translational control also plays an important role in the regulation of gene expressson. In this article, we wish to present a comprehensive and up to date overview of translational control of gene expression in eukaryotes. Much emphasis is given on animal systems where it is extensively studied. Various steps of protein synthesis have been discussed without which it would not be possible to discuss translational control.

Protein Synthesis in Eukaryotes

Translation is the process where amino acids are attached together on ribosomes in an ordered step-wise manner to form a polypeptide according to the nucleotide sequence of the messenger ribonucleic acid (mRNA). We are far from complete detailed understanding at molecular level as to how proteins are synthesized, though it is possible today to translate mRNA preparations easily into their respective polypeptides in test tubes (*in vitro*). Translation is a very complex process since it involves many components of the cells like mRNA, ribosomes, tRNAs, amino acids, aminoacyl-tRNA synthetases, ATP, GTP and various protein factors. The basic mechanism of translation is same in both prokaryotes and eukaryotes. Translation process in eukaryotes, which is more complex as compared to that of prokaryotes, is discussed in detail. However, the differences between prokaryotes and eukaryotes are mentioned wherever necessary.

Translation is a very systematic and sequential process that proceeds in three distinct steps called initiation, elongation and termination of the polypeptide chain synthesis.

Initiation

The initiation of protein synthesis involves binding of initiator met-tRNA (Met-tRNAi) to 40s ribosomal subunit followed by binding of mRNA and 60s subunit to form 80s initiation complex (80s. Met-tRNAi. mRNA) (1, 2). This step is much more complex than that in prokaryotes, and has been recognized as the main chain in regulation of translation. As many as nine protein factors, which are not integral part of ribosomes but associated with ribosomes called eukaryotic initiation factors (eIFs), are known to promote the formation of 80s initiation complex (1-3). All these factors have been purified and characterized by various laboratories. Some of the functional and structural characteristics of these factors are summarized in Table 1. However, it is known that only eIF–2, eIF–3 and eIF–5 are absolutely essential for translation of natural mRNAs whereas other factors (eIF–I, –4A, –4B, –4C, –4D, –4E and –4F) only lead to 2–3 fold stimulation of translation *in vitro* (1).

Initiation of translation can be divided into four distinct steps –(i) Ternary complex formation, (ii) binding of ternary complex to 40s to form 40s pre-initiation complex, (iii) binding of mRNA to 40s pre-initiation complex resulting in 40s initiation complex, (iv)binding of 60s to 40s initiation complex to form 80s initiation complex.

(i) Ternary complex formation is the binding of initiator tRNA (Met-tRNAi) to a protein factor, eIF–2, in the presence of GTP (1). Formation of binary complex (eIF2. GTP) has been claimed to be an intermediate step (4). eIF–2 can also form binary complex with GDP (5). However, this binary complex (eIF–2GDP) can not bind to Met–tRNAi. eIF–2 has been purified and characterized from a variety of systems by many investigators (6–9). The purified eIF–2 has a native molecular weight of 150,000, and has three non-identical subunits –$\propto$, β and Υ corresponding to molecular weights 38,000, 52,000 and 54,000 (5,7,10,11). Some laboratories could isolate eIF–2 from rabbit reticulocytes which has only two subunits (12,13). This β–less eIF–2 can form ternary complex and 40S complex. However, it is established that eIF–2 has three subunits *in vivo*, and the isolation of two subunit eIF–2 is due to limited proteolysis of eIF–2 by proteases during isolation (14) Initially, it was thought that $\propto$ and β bind to GTP and Met–tRNAi, respectively (13). Since β–less eIF–2 could form ternary complex, it was clearly established that β is not involved in Met-tRNAi binding, possibly Υ -sub unitbinds to Met–tRNAi. However, no function has be attributed to β subunit.

(ii) The next step involves the binding of ternary complex to 40S ribosomal subunit. For this purpose generation of 40S ribosomal subunit is required. Under physiological conditions, 80S ribosome is in dynamic equilibrium with its subunits (15). Hence, for the generation of 40S subunits, reassociation of 40S with 60S subunits should be prevented. A protein factor, eIF–3, which has high affinity towards 40S, binds stoichiometrically to form 40S. eIF–3, and thereby, preventing the joining of 60S subunit (16). On the basis of its function, eIF–3 is also called antiassociation factor. The ternary complex thus formed, as described earlier, is transferred to 40S subunit. This binding of ternary complex to 40S subunit is facilitated by eIF–4C (17) and stabilized by eIF–1.

(iii) In the following step, mRNA binds to 40S preinitiation complex to form 40S initiation complex. The mRNA binds to 40S subunit only eaftr Met–tRNAi is bound to 40S subunit (18, 19). This is in contrast to

Table 1. Characteristics of protein factors involved in initiation of protein synthesis of pro- and eukaryotes.

System	Nominclature	Native mol. weight X 10^{-3}	No. of subunits and mol. wt. in Kd	Function
Prokaryotes	IF-1	9	1	Binding of ternary complex to 30S
	IF-2	80	1	f-Met tRNA binding to 30S
	IF-3	23	1	Anti association of ribosomal subunits and mRNA binding to 30S
Eukaryotes	eIF-1	15	1	mRNA-binding to 40S
	eIF-2	150	3 (57, 54, 38)	Met-tRNAi binding to 40S
	GEF			
	(i) free	250	5 (32, 40, 57, 65, 80)	Promotes ternary complex formation, resucues Mg^{+2} inhibition, recycling of eIF-2
	(ii) complexed with eIF-2	450	8 (32, 40, 57, 65, 80, 54, 52, 38)	
	eIF·3	700	9 (140, 120, 110, 69, 47, 45, 37, 31, 28)	Anti association of ribosomal sub-units and stimulation of met-tRNAi binding to 40S
	eIF-4A	50	1	mRNA binding
	eIF-4B	80	1	mRNA bindng
	eIF-4C	17	1	40S formation and met-tRNAi binding
	eIF-4D	16	1	Methionyl puromycin formation
	eIF-4E	24	1	Cap recognition during mRNA binding
	eIF-5	150	1	Joining 40S initiation complex with 60S ribosomal subunit and release of initiation factors from initiation complex.

prokaryotic 30S initiation complex, where mRNA binding to 30S subunit precedes ternary complex binding (f-Met-tRNA. IF2. GTP) (1). It is believed that after mRNA binding to 40S complex, eIF-I repositions Met-tRNAi so as to get the anticodon in the right place with respect to initiation codon (20). This step of mRNA binding to 40S preinitiation complex is very important since the selection of mRNA occurs at this stage. The mRNA binding requires hydrolysis of ATP but how many ATP molecules are hydrolyzed per molecule of mRNA binding is not known (10). Moreover, the role of initiation factors (and/or discriminatroy factors, if any) in mRNA binding is not fully understood. eIF-1, -4A,-4B,-4C,-4E and-4F seem to be required in this step though exact function of these factors is not known (19, 21, 22). It is believed that most of these factors have a function in mRNA cap recognition or cap binding or both. The most intriguing question is that how ribosomes find the initiation site on mRNA in eukaryotes. It has been established that in case of prokaryotic mRNA, between 5′ end and the starting codon (AUG), there are non-coding sequences (Shine Dalgarno sequences) which are complementary to 3′ end of 16S rRNA of the 30S subunit (23, 24). The frequency of initiation of a particular mRNA is related to its potential ability for base pairing with ribosomal RNA. In eukaryotes also, there are non-coding sequences between 5′ end and starting codon (AUG) (25). However, no such complementarity has been reported with 3′ end of 18S rRNA of 40S ribosomal subunit. All cellular eukaryotic mRNAs have their 5′ end blocked by post transcriptionally added 7-methyl guanosine which is referred as cap structure ($m^7 G^{5'} ppp^{5'}$ Xp Y where X and Y denote nucleotides) (26). Recent experiments suggest that this cap at 5′ end is involved in initiation process (21, 27). Sonenberg *et al.* (21,27) have isolated a protein of Mr 25,000 that binds to cap hence it is named as cap binding protein (CBP). It has been shown that CBP binds at the 5′ cap of mRNA and enhances the translation of capped mRNAs, but not uncapped mRNAs (21, 27). CBP is now designated as eIF-4E. It is also believed that the importance of 5′ cap is to ensure that the translation begins at the 5′ end of the message. It has been proposed that 40S binds at 5′ end with help of CBP (eIF-4E) using the cap as a guide and proceeds along mRNA until it reaches the first AUG codon (25, 28). Involvement of ATP as energy source for 40S movement has been suggested (29). Secondary structure of the 5′ non-coding sequences is found to be very crucial for migration of ribosome (30-32). Apart from this, Kozak has recently demonstrated that the adjacent nucleotides at the 5′ end of AUG play a role in recognizing initiation codon (33, 34).

(iv) At the last step of initiation process, 60S subunit binds to 40S initiation complex to form 80S initiation complex. It requires three factors-eIF5, 4C and 4D. Out of these, eIF-5 catalyzes the hydrolysis of GTP and releases other initiation factors. This release of all initiation factors takes place at the time of 60S binding to 40S initiation complex (35). eIF-4C possibly stabilizes Met-tRNAi in the 80S complex (17). A schematic diagram of initation complex formation in eukaryotes is shown in Fig. 1. 80S initiation complex formation is accompanied by GTP hydrolysis and eIF–2 is released in the form eIF–2. GDP complex (5). In the presence of Mg^{+2} (which is required during all steps of initiation complex formation except ternary complex formation), eIF–2 has a high affinity for GDP, atleast 100-fold higher than that of GTP (36). However, for the formation of ternary complex eIF–2. GTP complex is needed because eIF–2. GDP cannot form ternary complex. Hence, it requires a mechanism for exchange of eIF–2 bound GDP with GTP. This exchange of GDP with GTP is catalyzed by another protein factor called GDP exchange factor (GEF). In the absence of GEF, eIF–2 would not be able to participate in second cycle as eIF–2 gets tied up as eIF–2. GDP. The ternary complex formation in presence of Mg^{+2} proceeds as follows:

$$\begin{aligned}
&\text{eIF-2. GDP} + \text{GEF} \rightleftharpoons \text{eIF-2. GEF} + \text{GDP}\\
&\text{eIF-2.GEF} + \text{GTP} \rightleftharpoons \text{eIF-2.GTP} + \text{GEF}\\
&\text{eIF-2.GTP} + \text{Met-tRNAi} \rightleftharpoons \text{eIF-2.GTP.Met-tRNAi}\\
\hline
&\text{eIF-2. GDP} + \text{GTP} + \text{Met-tRNAi} \overset{\text{GEF}}{\rightleftharpoons} \text{eIF-2.GTP.Met.tRNAi} + \text{GDP}
\end{aligned}$$

This GDP exchange reaction is analogous to exchange of GDP from elongation factor of EF–TU, of prokaryotes (37, 38) as shown in Fig. 2.

GEF and eIF–2. GEF complex have been purified to hemogeneity (39–44). Free GEF has a molecular weight of 250,000 with five non–identical subunits whereas eIF–2.GEF complex (Mr 450,000) when electrophoresced on denaturing gel shows eight bandes, three of which co–migrate with the three subunits of eIF–2 (5), GEF was first discovered by Ochoa's group (39, 40) and designated as ESP (eukaryotic stimulatory Protein). Later on, various investigators reported and used different names, e.g. stabilizing factor (SF) and inhibition reversal factor (RF) by London's and Gupta's groups (41, 42), eIF–2 stimulator by Clemens's group (43). However, recently Henshaw's group, for the first time, designated it as GDP exchange factor (GEF) based on its GDP exchange function (44).

Apart from the above mentioned initiation factors, three ancilliary factors (CoeIF–2A, CoeIF–2B and CoeIF–2C) have been described by Gupta's laboratory (2, 45, 46). Both CoeIF–2B and CoeIF–2C have been studied in crude form which resemble eIF–2.GEF in their properties and function. Hence, it was argued by some workers that the active factor in CoeIF–2B and CoeIF–2C preparations is not different from eIF–2. GEF (2). CoeIF–2A has been purified (Mr 25,000) which causes a moderate (2-3 fold) stimulation of ternary complex formation (46). Because of its low stimulatory effect on ternary complex formation, it was argued that eIF–2A may not be a factor essential for protein synthesis (2).

In contrast to eukaryotes, prokaryotes have only three initiation factors

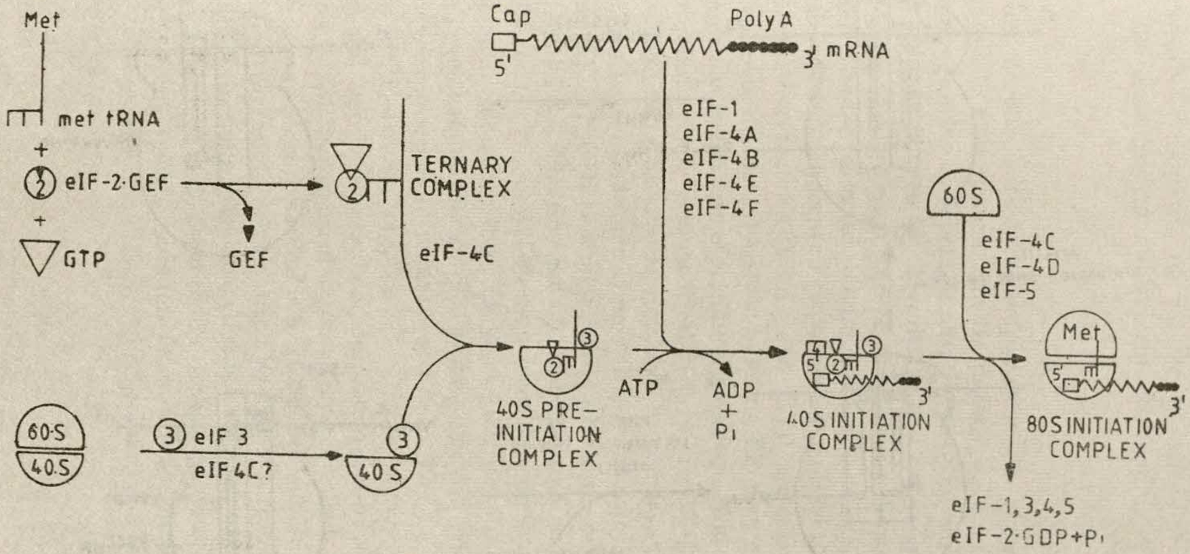

Fig. 1 Schematic representation of initiation complex formation in eukaryotes.

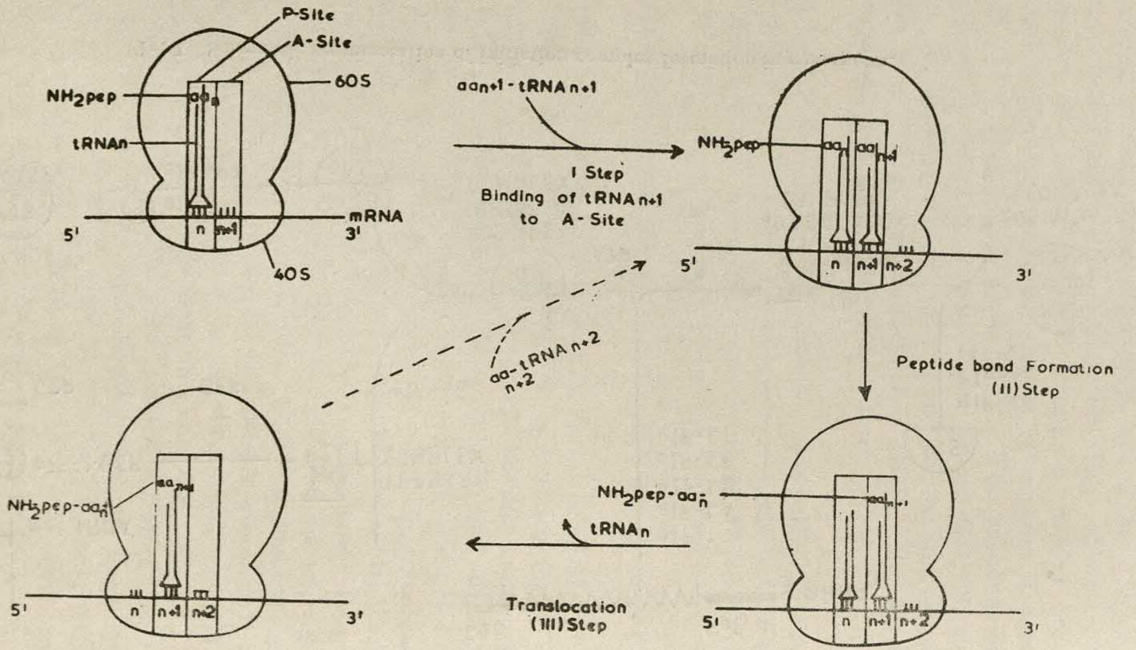

Fig. 2 : Schematic representation of the GEF catalytic cycle of ternary complex formation in eukaryotic polypeptide chain initiation (A) and the EF–Ts cycle of ternary complex formation in prokaryotic polypeptide chain elongation (B).

IF–I, IF–2 and IF–3 (1). IF–3 like eIF–3 helps in generatin of 30S subunits and also promotes binding of mRNA to 30S subunit. Like eIF–2, IF–2 forms ternary complex with GTP and initiator-fMet–tRNA f which in turn binds to 30S.mRNA complex. As we see, in case of prokaryotes, binding of mRNA to 30S ribosomal subunit precedes the binding of ternary complex.

Elongation

Formation of 80S initiation complex sets the stage for polypeptide chain elongation. This process can be defined as sequential addition of amino acids to growing polypeptide as directed by mRNA. Elongation involves the relative movement of mRNA and ribosomes. The process of polypeptide chain elongation can be conveniently discussed in three steps.

First step is codon directed binding of aminoacyl–tRNA to the vacant aminoacyl site (A–site) adjacent to an occupied peptidyl site (P–site) on 60S subunit. Elongation factor–I (EF–I) and GTP are required for this step (38). The second aminoacyl–tRNA binds to EF–I ∝ and GTP to form a ternary complex as in the case of initiation process. However, unlike eIF–2, EF–I∝ can bind to any aminoacyl–tRNA (47). EF–I consists of three subunits ∝, β and Υ. EF–I∝ is involved in ternary complex formation while EF–I β is involved in recycling of EF–I∝. After one cycle, EF–I∝ is released as EFI∝. GDP complex. The exchange of GDP with GTP is catalyzed by EF–I β. The function of Υ subunit is not known. The ternary complex (EF–I∝.GTP. aa–tRNA), thus formed, is then transferred to A–site of 60S ribosomal subunit. In prokaryotes, this step is similar to eukaryotes where EF–T (elongation factor–T), which has two subunits EF–Tu and EF–Ts, functions in a similar manner as EF–I. EF–Ts, like EF–I β, helps in recycling of EF–Tu (38) Fig. 3..

The second step is peptide bond formation between aminoacids in 'A' and 'P' sites. This is catalyzed by an enzyme called peptidyl transferase (48) which is an integral part of 60S ribosomal subunit. The third step is translocation process by which peptidyl–tRNA is translocated from 'A' to 'P' site to allow incoming aminoacyl tRNA to enter the vacant A–site with concomitant release of discharged tRNA from the P–site. This translocation process requires eukaryotic elongation factor–2 (EF–2) and GTP (49). EF–2 corresponds to prokaryotic EF–G. A schematic diagram of elongation is shown in Fig. 4.

Termination

Termination procees releases the completed polypeptide chain from the ribosomes and mRNA complex. This is directed by one of the three terminator codons (UAA, UAG, UGA). When a termination codon is reached by ribosome, a termination factor callod release factor (RF) catalyzes the hydrolysis of the bond between completed polypeptide chain and tRNA

(50). This event requires hydrolysis of GTP to release mRNA from the ribosomes (51).

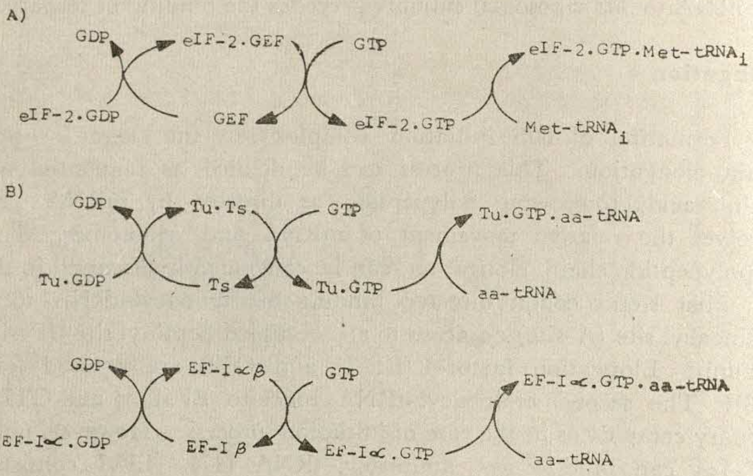

Fig. 3. Schematic representation of FF-I recycling during polypeptide chain elongation in eukaryotes.

Fig. 4. A schematic model for polypeptide chain elogation.

In contrast to eukaryotes, where there is only one RF which recognizes all the three termination codons, prokaryotes have two release factors (RF–1 and RF–2) with different codon specificities. RF–1 recognizes UAA and UAG, whereas RF–2 identifies UGA and codons. In addition to RF–1 and RF–2, another factor called RF–3 was also reported. RF–3 itself does not have any release activity but stimulates the activity of RF–1 and RF–2 (50). The process of termination differs from initiation and elongation processes since the codon recognition molecules are not tRNA but proteins.

Regulation of Translation

In prokaryotes, where transcription and translation processes are coupled, gene expression is mainly regulated at the level of transcsiption. Moreover, mRNAs have a short half life. However, the situation is entirely different in eukaryotes. Transcription and translation processes are spatially separated by nuclear membrane. Moreover, eukaryotic mRNAs are metabolically stable (*e.g.* mRNAs are stored for months/years in embryos) and exhibit some unique structural feature that are absent in prokaryotes. Eukaryotic mRNAs have their 5' end blocked by post–transcriptionally added 7–methyl guanosine which is referred as 'cap' structure (26). And 3' end of mRNA contains a long stretch of poly (A) which is also added post transcriptionayll by an enzyme called poly (A) polymerase. Moreover, the non-coding sequences of mRNA (introns) are removed by a process called 'splicing'. The stability of mRNAs, large number of initiation factors with their complexity, spatial separation of transcription and translation and increased complexity of ribosomes clearly suggest the existence of finer regulation of translation in eukaryotes.

Regulation of gene expression by translation control is known to function in many instances (Table 2). There can be two kinds of translational control, namely, non-selective and selective control (52). In case of non-selective control, translation of all the messengers is affected to the same extent. Selective translation control means a set of messages from a variety of different mRNAS are selected for translation with same or differential efficiency.

In non-selective translational control, alteration of some crucial components of the translational machinery turns on or off the total protein synthesis. A good cxample of this kind of control is regulation of polypeptide chain initiation by phosphorylation of initiation factor 1-2 (eIF-2) in reticulocytes and other eukaryotic cells (2, 53).

Table 2. Some instances where regulation of gene expression occurs at the level of translational control.

1. Viral inhibition of host protein synthesis.
2. Expression of latent mRNA during embryonic development and cell differentation.
3. Coordination of ribosomal protein synthesis.
4. Regulation of early and late gene expression during viral replication.
5. Rapid adjustment of protein synthesis with energy and nutritional status.
6. Establishment of the antiviral state by interferon.
7. Coordination of heme and globin biosynthesis during retyculocyte maturation.

Translational Control in Animals

Regulation of protein synthesis at translational level in animals is relatively well studied and most of the information comes from reticulocytes and their lysates (2). Rabbit reticulocytes and their lysates have been chosen as model system to study the translational regulation since these are non-nucleated cells and there can only be translational control. Translational control in eukaryotes appears to operate at initiation of polypeptide chain and this may explain why eukaryotic initiation factors are numerous and structurally more complex than their prokaryotic counter parts.

In reticulocytes, hemoglobin constitutes the bulk of the proteins synthesized. The synthesis of globin occurs only in the presence of heme, the porostetic group of hemoglobin. In rabbit reticulocyte lysates, translational activity depends on the presence of hemin (54, 55). If the lysate is not supplemented with hemin the protein synthesis shuts off within 5 to 10 minutes as shown in Fig. 5. Later investigations from various laboratories have firmly established that hemin deficiency leads to the activation of an inhibitor of polypeptide chain initiation which exists in reticuloyte lysates in an inactive form (56, 57). Presence of hemin blocks the conversion of inactive inhibitor to active inhibitor (pro-inhibitor hemininhibitor). This inhibitor is called hemin-controlled inhibitor (HCI). HCI has been purified and characterized by several laboratories (58–61) as a cAMP-independent protein kinase (eIF-2-kinase) which specifically phosphorylates the α subunit (38, 000 dalton) of eIF-2.

(i) **HCI Activation**: The activation of HCI accompanies its phosphorylation (61–63). Inhibition of activation by hemin blocks the phosphorylation of the HCI. The question regarding the activation of inhibitor in absence of hemin, is not clearly understood. In 1977, it was proposed that HCI is activated by phosphorylation catalyzed by cAMP-dependent protein kinase

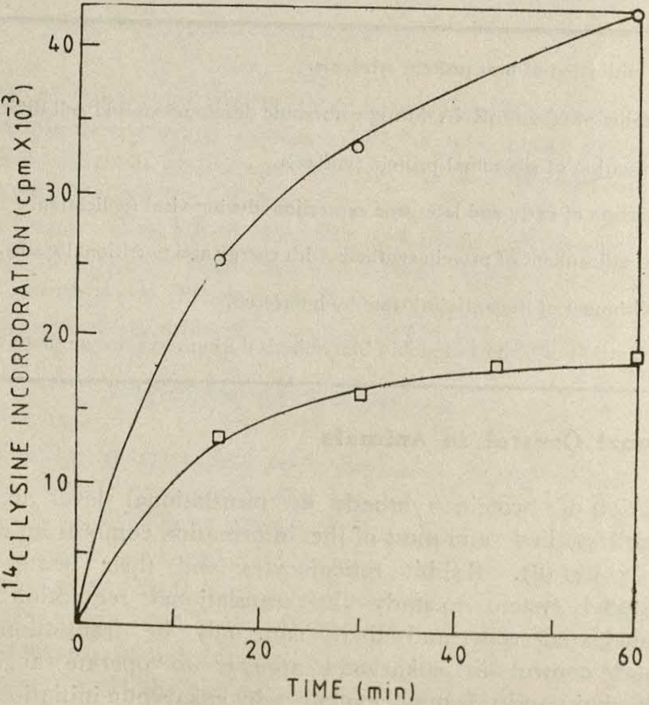

Fig. 5. Protein synthesis in the reticulcyte lysate in the absence (□-□) and presence (o-o) of 34 μM hemin.

(64–66), since cAMP-dependent protein kinase converts inactive HCI to active HCI and consequently inhibits translation. Hemin blocks the phosphorylation of HCI by inhibiting activity of cAMP-dependent protein kinase by inhibiting cAMP-induced dissociation of 'R' and 'C' subunits of cAMP-dependent protein kinase which is essential for its activity (64–66). However, this model had to be abandoned because the inhibition by cAMP-dependent protein kinase is not due to its phosphorylating activity (67, 68) but due to a heat stable contaminant of Mr 68, 000 which is also a strong inhibitor of inititation (69). This heat stable inhibitor also converts pro HCI to HCI by some unknown mechanism. In addition, oxidized glutathione (GS–SG), thiol reagent such as N-ethylmaleimide, calcium and phospholipids are known to activate HCI (2, 70, 71). However, the mechanism of activation is not known.

(ii) **Mode of Action of HCI** : The molecular mechanism of inhibition of translation by HCI has been studied by many laboratories. As mentioned earlier, there is a correlation between the hemin deficiency in reticulocyte lysates and phosphorylation of ∝-subunit of eIF–2 (59–62). The phosphorylation is catayzed by active HCI, a cAMP-independent protein kinase which is very specific for eIF–2. It has become clear only recently as to how eIF–2 (∝ P) shuts off protein synthesis. eIF–2 (∝ P) can form ternary complex and 40S initiation complex formation (62). It has been proposed that phosphorylation of eIF–2 interferes in its recycling (2). As mentioned earlier, eIF–2 is released as eIF–2.GDP, after 80s initiation complex formation (72). Before eIF–2 participates in another cycle, GDP of eIF–2.GDP complex should be exchanged for GTP for which GEF is required (also see earlier discussion on initiation process). This exchange takes place *via* eIF–2.GEF intermediate. It was earlier believed that phosphorylated eIF–2, eIF–2 (∝ P), loses the affinity for GEF. Therefore, it is unable to dissociate GDP from eIF–2.GDP which is required for recycling of the initiation factor (5). However, later experiments have shown that GEF can interact with phosphorylated eIF–2 and has a high affinity towards phosphorylated eIF–2 (73, 74). Because of this observation, it is strongly believed that GEF, since it has high affinity to eIF–2 (∝ P), is immobilized as eIF–2 (∝ P).GEF hence GTP cannot bind to it. eIF–2 (∝ P).GEF has also been detected *in vitro* reticutocyte lysate translation assays with excess of GDP (75). Hence, the translational inhibition is due to sequestering of GEF in the form of eIF–2 (∝ P).GEF that is not readily dissociable. This renders GEF unavailable to catalyze eIF–2.GDP dissociation thereby blocking the recycling of eIF–2 (73, 74). Because of this reason, phosphorylation of 20–30% of total eIF–2 will fully inhibit translation in hemin deficient lysates (76).

(iii) **DAI Activation** : Addition of low amounts of double-stranded RNA to hemin containing reticulocyte lysates activates HCI like inhibitor called dsRNA activated inhibitor (DAI) (62, 77). Like HCI, DAI is also cMP–

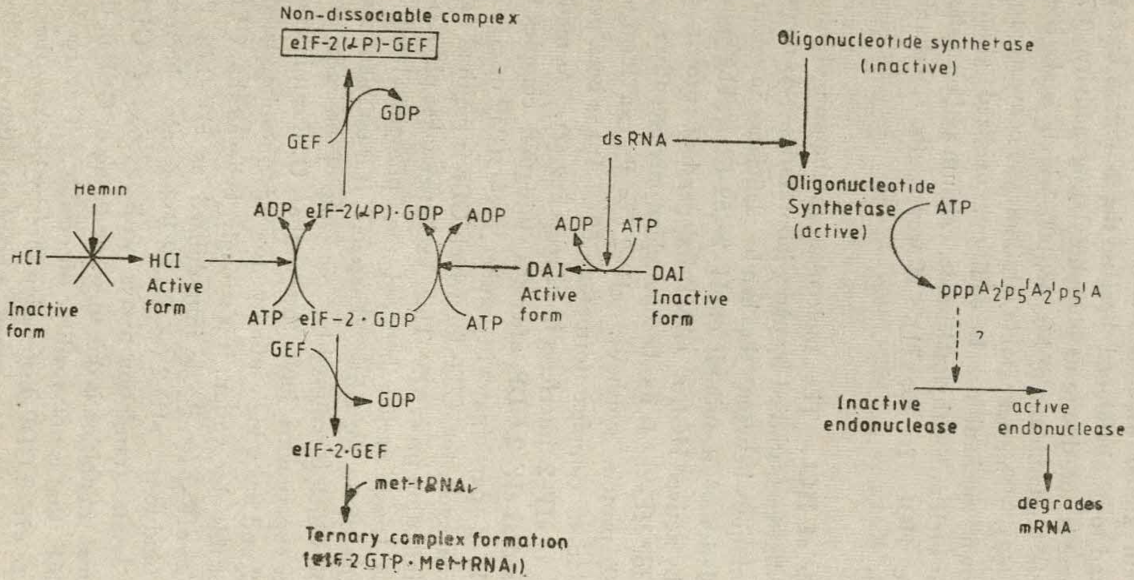

Fig. 6. A model for the regulation of translation by HCI and DAI
HCI = hemin controlled inhibitor
DAI = double straded RNA activated inhibiter
eIF-2 (P) = Phosphorylated eIF-2
GEF = GDP exchange factor
dsRNA = double stranded RNA.

independent protein kinase (eIF–2 kinase) which phosphorylates small subunit of eIF–2 (78, 79). Unlike HCI, DAI is present in association with ribosome and also phosphorylates histone. Inactive DAI is a constitutive in lysate and is activated by dsRNA. As in the case of HCI, activation of DAI involves its phosphorylation by dsRNA. However, how dsRNA phosphorylates DAI is not known. There are two possibilities: one, the involvement of dsRNA dependent protein kinase, another is the activation of DAI by autophosphorylation in presence of dsRNA and ATP. The mode of activation is shown in the following steps:

$$\begin{array}{l} \text{Inactive DAI} + \text{dsRNA} \longrightarrow \text{Inactive DAI} + \text{DAI.dsRNA} \\ \qquad\qquad\qquad\qquad\qquad\qquad \Big\downarrow \begin{array}{l}\text{ATP}\\ \text{ADP}\end{array} \\ \qquad\qquad\qquad\qquad\quad \text{DAI}{\sim}\text{P (active)} + \text{DAI.dsRNA} \end{array}$$

This model assiumes that DAI molecules activated by dsRNA binding can phosphorylate other free DAI molecules but not themselves (80).

Besides the activation of DAI, incubation of reticulocyte lysates with dsRNA and ATP also gives rise to the formation of a potent heat stable translational inhibitor, a novel oligonucleotide (pppA2 p5'A2'p5'A) that inhibits protein synthesis at exceedingly low concentrations possibly by activating an endonuclease that degrades both cell and viral mRNAs (81).

Treatment of mouse L cells and ascites cells with antiviral agent, interferon (interferons are glycoproteins synthesized by various animal cells as a result of viral infection or of treatment with interferoninducers), induces inactive DAI and pppApApA synthetase. These are activated in the presence of dsRNA as described in the case of reticulocyte lysates. These interferon induced enzymes are present in constitutive manner in reticulocytes (82,83). In addition, interferon also induces the activation of endonuclease which degrades mRNAs. This type of regulation seems to play an important role in inhibiting growth of the virus and virus infected cells since viruses induce interferon in animal cells.

The control of protein synthesis in reticulocytes by HCI and its activation by heme deficiency seems to be purposeful regulatory mechanism of globin synthesis to ensure that globin is synthesized only when heme, its prosthetic group, is available. Protein kinases that can inhibit translation have been isolated from various eukaryotic systems other than reticulocytes. These include Ehrlch ascites tumor cells (84), Friend Leukemia cells (85), Rat liver (86), *Artemia salina* (87), Krebs ascites cells (88, 89), wheat germ (87, 90), Barley (91). Reticulocyte lysates have protein phosphatases that dephosphorylate eIF–2 (α p), active HCI (HCI-p) and DAI (DAI·p). Hence, the level of phosphorylation of eIF–2 (α p) subunit is dependent on the balance between phosphorylation and dephosphorylation process of eIF–2 and its protein kinases (92). Besides the phosphorylation of α

subunit of eIF–2, eIF–2 β subunit and other initiation factors (eIF–3, eIF–4B and eIF–5) can also be phosphorylated by other protein kinases. However, the physiological significance of this phosphorylation is not known as catalytic properties of these factors are unaltered after phosphorylation (2, 93).

(iv) **Small Molecular Weight RNAs Role in Translation** : Small molecular weight RNAs have been implicated in the regulation of translation. Fuhr and Natta (94) had, for the first time, isolated an RNA molecule in reticulocytes of β– thalassemia that stimulated β–globin synthesis and inhibited β –globin synthesis. However, Dionne *et al.* (95) isolated another small RNA from the same system that inhibits specifically initiation of translation.

Heywood's group has isolated two small RNAs, referred as translational control RNAs (tcRNA) from chick embryonic muscle poly somes (polysomal tcRNA) and free mRNP particles (mRNP–tcRNA) (96, 97). mRNP–tcRNA is believed to play a role in conservation of myosin mRNA by inhibiting its translation. Unlike polysomal tcRNA, mRNP–tcRNA inhibits translation of only myosin mRNA but not other mRNAs (98, 99). It is proposed that mRNP–tcRNA hybridizes with the poly (A) sequence of the myosin mRNA and possibly altering its confirmation thus leading to inaccessability of mRNA for translation (99). Since mRNP–tcRNA does not affect the translation of other Poly (A)–mRNAs, it is believed that a few short sequence at 3' and 5' ends of tcRNA, that are complementary to certain sequences of myosin mRNA are involved (99). In contrast to this report, Pluskal and Sarkar (100) have isolated another cytoplasmic small molecular weight RNA from the same system that inhibits initation of translation indiscriminately by preventing mRNA binding to the 40S ribosomal subunit (101). Based on their observation, they proposed that this RNA does not bind to mRNA but interacts directly with 40S probably by competing with mRNA for a common site.

Two species of tcRNAs from *A. salina* – one inhibitory and the other stimulatory to translation *in vitro* which play a role in development have been isolated (102). Inhibitor RNA is found both in dormant and developing stages while activator is present only in latter stage. Inhibitor RNA inhibits translation specifically at elongation step by interferring with EF–1 dependent ribosome binding of aminoacyl–tRNA. It is proposed that inhibitor RNA plays a role in conserving mRNA which will be utilized during development stage. At the onset of development of embryo, the activator RNA level goes up, hybridizes with inhibitor RNA and inactivates. Hence, the level of inhibitor and activator RNAs will influence the onset af development.

Various agents that inhibit protein synthesis in general and their mode of action have been discussed so far. Another mechanism of regulation of protein synthesis is at the level of selection of mRNA for translation by the translational apparatus. This is an important mode of regulation because in eukaryotes, a variety of mRNAs are known to exist in a cell at a given time. Therefore, selection of mRNA for translation depends on the requirement of the cell/tissue. It is also very crucial during development and differentiation of cells/tissues. There are evidence that some initiation factors might have a role in this process. Heywood and co-workers have found a factor (possibly eIF-3) in ribosomal wash of chick embryonic muscle which stimulates the translation of only myosin mRNA in a cell free system (103, 104).

Since mRNA recognition occurs at the binding of mRNA to the 40S subunit, the initiation factors involved at this step may play a role in mRNA selection. The importance of the cap binding or recognizing initiation factor (s) in selection of capped RNA for translation has been shown in viral infected cells (22, 105, 106).

Translational Control in Plants

Not much is known regarding the mechanism (s) that regulate gene expression at the translational level in plants. The question whether the translational control mechanisms that occur in animal systems can be extended to plant systems is yet to be answered. Information regarding translational control mechanisms in plants, which is lacking, is essential in order to see whether there exist common mechanism (s) that control translation in phylogenetically apart plants and animals. Because of paucity of information and potential scope, plant systems are good candidates for future research.

Many plant seeds contain stored messenger RNAs (107–109). These stored mRNAs are synthesized during embryogenesis and utiltized during germination. This kind of temporal separation of transcription and translation processes, which appears to be characteristic of early development in many organisms, indicates the possible existence of fine regulatory mechanism (s) at the level of translation. Hence, they serve as good model systems to study the gene expression at the translational level.

Rychlik's group (110, 111) purified a cAMP independent protein kinase from wheat germ extract which inhibited the translation of some exogenously added bromomosaic virus mRNA. They have also shown that the observed inhibition is due to arrest of 80S initiation complex formation without affecting 40S initiation complex formation. Hence, they suggested that this enzyme acts differently from hemin controlled inhibitor (HCI) of reticulocyte lysates. Phosphorylation of two ribosomes associated proteins of molecular weight 32,000 and 76,000 is believed to be the cause of inhibition (111). Presence of translation inhibitor analogous to HCI has also been reported in wheat germ (87). Later, a partially purified cAMP independent protein kinase from wheat germ called wheat germ inhibitor (WGI) has been shown to inhibit protein synthesis. A close resemblance between HCI and WGI has been shown. It was reported that WGI, like HCI, phosphorylates ∝-subunit of eIF–2 (90).

Recently, in our laboratory, a cAMP independent protein Kinase has been purified from barley embryos which inhibits translation (91). The molecular mechanism of inhibition is not known. However, the endogenous substrate for this protein kinase is not eIF–2. It has been shown that a protein of molecular weight 52,000 is involved (112). The involvement of this protein in protein synthesis initiation is imperative as the enzyme inhibits inttiation of translation. In addition to this protein inhibitor, barley embryos contain a small molecular weight RNA (smaller than tRNA) that inhibits initiation of translation (Fig. 7). However, the molecular mechanism of inhibition of this inhibitor is not known (113).

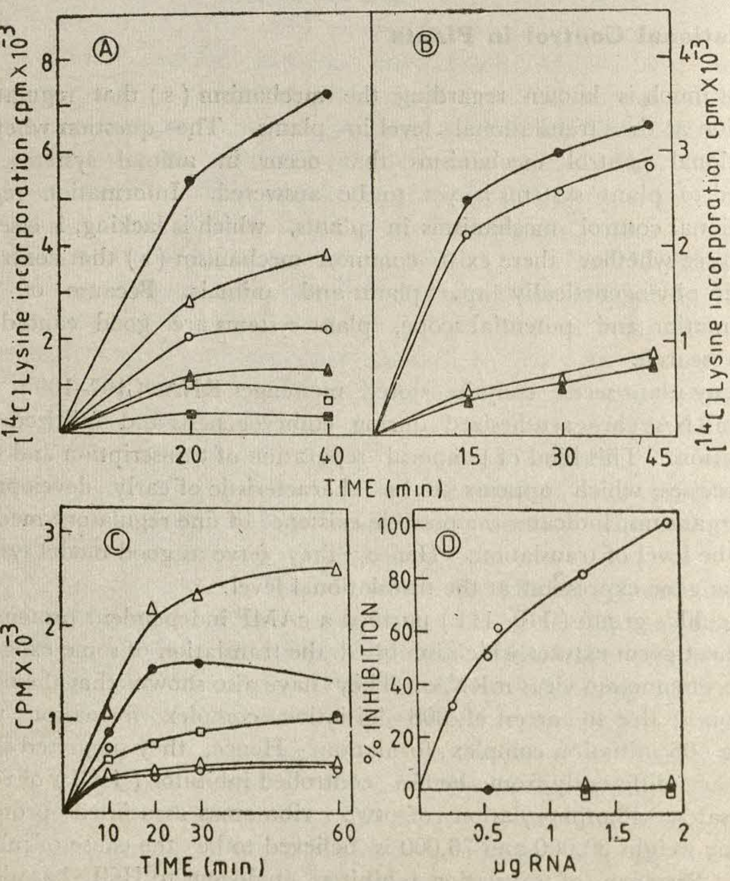

Fig. 7. Inhibition of translation by protein kinase (A and B) and iRNA (C and D) isolated from barley embryos.

A. Without hemin (....), with 3 µM hemin (....), hemin plus 0.7 µg PK (....), hemin plus 1.4 µg PK (....), hemin plus 3.6 µg PK (....), hemin plus 7.3 µg PK (....).

B. Without hemin (....), with 30 μM hemin (....) hemin plus 2.2 μg PK (....), hemin plus 2.2 μg of heat treated PK (....).

C. Without hemin (....), 20 μM hemin (....), plus 0.64 μg iRNA (....), hemin plus 1.29 μg iRNA (...), hemin plus 1.92 μg iRNA (....).

D. Without hemin (....), with 20 μM hemin (....), hemin plus alkali digesied RNA (....), hemin plus Pancreatic RNAse treated iRNA (....).

Translation Control in Prokaryotes

Regulation of gene expression at the translational level in prokaryotes is known to occur atleast in a few instances. In prokaryotic mRNAs, between 5′ end and initiation codon, AUG, there are non-coding sequences (Shine -Dalgarno sequences) which are complementary to the 3' end of 16S rRNA of 30S subunit (23, 24). Presence of six to eight purine rich nucleotides (ribosome binding site) upstream initiation codon, determines the efficiency of mRNA translation. In addition to these sequences, the position of ribosome binding site is also important. mRNA with ribosome binding sequence cetered eight nucleotides upstream inditiation codon are translated more efficiently. Positioning of the ribosome binding sequences either closer or further away from the AUG codon greatiy lowers the translation activity.

Synthesis of ribosomal proteins (r-proteins) occurs only in presence of r-RNA. The regulation occurs at the level of translation. If the rate of synthesis of r-proteins is more than that of r-RNA synthesis, some key r-poteins bind to Shine-Dalgarno sequences of mRNA specific to r-protein, thus preventing their translation. This kind of mechanism operates to control excess synthesis of r-proteins.

Recently, involvement of small RNA transcripts in the regulation of translation of some mRNAs in prokaryotes has been reported. The synthesis of an outer membrane protein (Omp F) of *E. coli* is regulated by a small RNA transcript, called mRNA-interfering-complementary RNA (mic RNA). This 174 base mic RNA is complementary to 44 bases of the non-coding region and 28 bases of coding region at the 5' end of Omp F mRNA. The hybridization of mic RNA with Omp mRNA results in prevention of translation of OmpF-mRNA (114). In another case, the synthesis of transposase of Transposon 10 (Tn_{10}) is controlled by a small RNA transcript, which has 36 base pairs complementary region with transposase mRNA, in a manner similar to mic-RNA (115, 116).

New Ways to Mutate Genes by Using Anti-Sense RNA

Recent results show that translation of mRNA can be prevented by introducing RNA complementary to that mRNA (i. e., anti-sense RNA) into cells (117, 118). Douglas Melton has shown that translation of injected β -globin mRNA in frog eggs is blocked if RNA-complementary to the β-globin mRNA is injected prior to globin mRNA. Interestingly, translation of β-globin mRNA is not blocked by anti-sense RNA for histone mRNA indicating that the effect is specific (118). It is believed that anti-sense RNA hybridizes to globin mRNA and blocks translation. Anti-sense RNAs for globin and histone genes were synthesized *in vitro* by using recombinant DNA techniques. Another group also reported the ability of anti-sense RNA to inhibit gene expression in cultured cells. Injection of plasmids that contain thymidine kinase (TK^+) gene into TK^- mouse cells (mutant) resulted in the expression of TK. However, injectin of constructed plasmid that can transcribe RNA complementary to TK kinase along with plasmids containing TK showed substantial reduction in the expression of TK (119).

The ability of an mRNA to form a stable duplex with its anti-sense RNA offers a potentially powerful mode for manipulating gene expression artificially. It may soon be possible to turn off the expression of specific genes in higher organism by anti-sense RNA of the same gene, which is nothing but production of gene mutation at our will, which otherwise is not possible with known technology. It is speculated that anti-sense RNA may be of use in gene replacement therapy though it would take long time to realize this potential application. However, studies with anti-sense RNAs offer a great scope in order to study gene function.

Conclusion

Even after 25 years of research on protein synthesis and its regulation, we are at the initial stage in understanding these comlex processes. Initiation of translation appears to be rate limiting step in protein synthesis in most systems and at which most translational control mechanism (s) operate. It is believed by many in the field that initiation of translation is regulated by modification of eIF-2. However, we cannot, possibly, argue that similar mechanism operates in all other spstems since most of these studies concerning eIF-2 phosphorylation are carried out in reticulocyte lysates. Moreover, efforts to study translational regulation in various other tissues are severely hampered as most of the tissues for some unknown reasons, yleld poor cell-free translation system. It may be possible that different kinds of regulatory mechanism (s) operate in different tissues (tissue specific regulatory mechanisms). Further more, even in the same tissue different mechanisms may operate depending on the circumstances. Recent studies on the involvement of small molecular weight and antisense RNAs indicate the possible existence of various other regulatory mechanisms. Moreover, our knowledge regarding selective translational control is very meagre. In principle, translational regulation might also occur at any point in the whole process of translation.

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3

PALAEOBIOLOGY OF DINOSAURS : RECENT STUDIES

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Introduction

Although dinosaurs have been singled out from a vast array of ancient reptiles to receive special attention by naturalists, palaeontologists and laymen for a long time, precise knowledge about them is only about 150 years old. They ruled the earth for an astonishing length of 140 million years. Teeth and fragmentary bones of *Iguanodon, Megalosaurus and Hylaesaurus* were described in 1930's in England without realising that they were dinosaurian. It was left to Richard Owen, a Hunterian Professor of Anatomy at the Museum of the Royal College of Surgeons, to coin the word '*Dinosauria*' (meaning 'terrible lizard') in 1842. The word was purely descriptive term to designate the large fossil reptiles that were being recognised in England, and was not meant, to be taken literally. In the early years of palaeontological research, however, the Dinosauria was regarded as a natural group of reptiles. As knowledge expanded about them, it became apparent that the term includes two distinct reptilian orders. In recent years, with the vast increase in our knowledge of dinosaurs, it has been realized that all dinosaurs were neither terrible nor giants. A large number of them were herbivores and quite a few of them were quite small, some as small as a hen.

As examination of the history and literature related to the dinosaurs reveals three phases leading to the development of current status of knowledge about dinosaurs. The early phase during the 19th and early part of the 20th century may be called a phase of Discovery in which dinosaur sites were discovered, fossils were excavated and transported to research organisations and eventual taxonomic assessments were made. This activtiy was largely confined to Europe and the U.S.A. The second phase which continued until early seventies concentrated on intensive research work leading to release of popular knowledge and stet mounting of skeletons and skeletal restorations in Natural History museums. *In-situ* display of dinosaur bones at the Dinosaur National Monument in Utah (U.S.A.); visited by several thousand visitors yearly, was an important step in the dissemination of knowledge about dinosaurs. It bridged a gap in the mind of the visitor between a mounted skeleton, the site of discovery and excavation of bones. The display of dinosaur skeletons stimulated the imagination of people from all walks of life which unfortunately also led to the release of distorted and clearly unscientific stories about dinosaurs. This second phase or the phase of Displays, therefore, also attempted to release authentic and accurate information of dinosaurs as a result of intensive research. Lists of valid genera (White, 1973; Olshevsky, 1978) of dinosaurs and numerous textbooks and popular books on dinosaurs were published during this phase. Some 500 valid species belonging to about 300 dinosaur genera of the two orders of dinosaurs were recognised during seventies.

The third or the Modern Phase, started during the seventies, has added a new dimension by upturning a number of old concepts about the dinosaurs. This had led to extensive research in related fields as a result of new and challenging ideas. Dinosaurs so long considered similar to living reptiles were suggested to be active, perhaps even warm-blooded animals, comparable to modern mammals and birds. Furthermore, new evidences about the phenomena of dinosaur-extinction in the form of extra-terrestrial events led to a new and fertile field of research. It has also been suggested that although dinosaurs shared a common ancestry with crocodiles, their lineal descendents may be birds. The important landmarks which ushered in the modern phase are the publication of several books and monographs in addition to numerous research papers. The most important among these are :(1) *Hot-blooded dinosaurs* by Desmond Morris in 1975, (2) *Dinosaur renaissance* by Robert Bakker in 1975, (3) *A new look at the dinosaurs* by John Ostrom in 1978, (4) the holding of a Symposium and publication of its Proceedings as "*A cold look at warm blooded dinosaurs*" by the American Association for the Advancement of Science (AAAS) in 1980 and (5) organisation of a Workshop and publication of its Proceedings (as *Syllogeus* No. 39) on '*Cretaceous-Tertiary extinctions and possible terrestrial and extraterrestrial causes* (K-Tec II)" by the National Museum of Natural Sciences, Ottawa in 1982. The extent of research and new dimensions can be partly gauged from the mere fact that AAAS Symposium refers to over 600 and Ottawa Symposium to about 125 papers, mostly published during last 2-3 decades.

The present article is heavily based on published papers during last 2-3 decades to summarise a part of the new knowledge about the palaeobiology of dinosaurs. The underlying idea is not to survey (which would have taken a much longer space) but to indicate briefly certain aspects of the new ideas. The purpose of this exercise is two-fold. *Firstly*, India has already gone through the first two phases of discovery, exploration and display of dinosaurs and even though much remains to be done in this respect, it is time to take up studies related to the Modern Phase. *Secondly*, the new discoveries have ushered in a multi-disiplinary approach in which the biologists, biochemists, environmentalists and even engineers are playing a key role. The dinosaur-research, which in early phases was the domain of explorers, field geologists and stratigraphers, requires the collaboration and joint efforts by other disciplines in the Modern Phase. This essay, therefore, is addressed to biologists, rather than palaeontologists to develop an awareness and interest for future research in this and related areas of dinosaur studies.

Dinosaur Classification and Discoveries

Over 500 different species of dinosaurs are so far known and further remains are continually discovered. Many forms are known from isolated bones or teeth which prove the existence of different species but do not provide sufficient evidence to allow a reconstruction of complete skeleton. Some species are distinguished on minor anatomical features of particular bones which wouid not be observable in life restorations.

Basis of Classification

The basic reptilian method of locomotion is a sprawling gait. Dinosaurs are distinguished from all other reptiles by the manner in which they stood and walked. The limbs were held vertically giving them an erect posture and gait akin to birds and mammals. Reptilian evolution reached its peak in the dinosaurs. The posture is also reflected in the structure of their limbs and limb girdles, particularly pelvic girdle. As with all reptiles, the pelvic girdle consists of three elements : a dorsal ilium which is fused to the sacral vertebrae and ventrally an anterior pubis and posterior ischium. Femur articulates at the cup-shaped hollow or acetabulum where three pelvic bones meet. In dinosaurs, with the limb held vertically, there is on force exerted in this region and in consequence there is no longer a solid wall of lone but instead an open space or fenestra.

The earliest members of the dinosaurs were bipedal, walking on hind legs with the body pivoted at the hips. The structure of the pelvis was of critical importance in the functional anatomy and evolution of these reptiles. In this respect the development of the dinosaurs as two separate orders is exellently indicated by a quite distinct form of the pelvis. The

Saurischia, so named because the pelvis in general is similar to many other reptiles, have a triradiate structure. It has a deep, rather plate-like bone, the *ilium*, at the top, connecting with some sacral vertebrae, a rod-like *pubis* extending down and forwardly, and a rod-like *ischium* extending down and backwardly (Fig. 1A). The Ornithischian derive their name from the fact that the pelvis is strikingly similar in some respects to the pelvis of birds. It consists of a large plate-like *ilium*, rather more elongated than the ischium of saurischians, a rod-like *ischium* and a rod-like *pubis*; but in this case the pubis is rotated backwardly so that it is parallel to the ischium. Often the front part of the ornithischian is expanded into a large process that extends forwardly under the front part of the ilium (Fig. 1B).

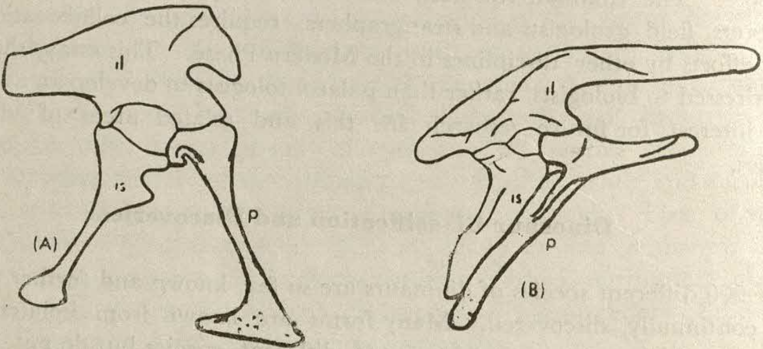


Fig. 1. The pelvis in the two dinosaurian Orders. A, Saurischian pelvis, with a forwardly directed pubis: B, Ornithischian pelvis, with a pubis parallel to the ischium (from Colbert, 1962) ; *il.*-*ilium*, *is.*-ischium. *p.*-pubis.

In addition to the structure of the pubis, there are other diagnostic characters in dinosaurs related to their jaws and teeth. In saurischian dinosaurs the teeth either extended around the margins of the jaws, above and below, or were limited to the front portion of the jaws. The ornithischians had lost the teeth in the front of the mouth in both upper and lower jaws, and generally this portion of the skull and jaw took the shape of the beak of some sort. These dinosaurs also had a new single bony element on the front of the lower jaw, the *predentary*, forming the lower portion of the cutting beak. The teeth, limited to the sides of the jaws, were often highly modified for cutting and chewing vegetation since all ornithischians were herbivorous.

Dinosaur Family Tree

Although Mesozoic era is regarded as the Age of Dinosaurs, it was not until late in the Triassic times that the first dinosaurs appeared. They became well established during the later phases of Triassic history, but even so they were not the dominant land animals, that they were during the Jurassic and Cretaceous periods. The first dinosaurs were saurischians, typified by bipedal carnivorous types such as *Coelophysis* and grouped as Theropods. During late Triassic a further saurischian group of dinosaur made its appearance which had more massively constructed limbs and bodies and generally seem to have walked on all fours. These four-footed early dinosaurs are known as Prosauropods. These represent the first group of dinosaurs to become plant eaters. This group is notable for the tremendous increase in size from 2 m long to the largest it reaching a length of 12 m. The saurischian history extended right upto the close of the Cretaceous times. The other Order, Ornithischia, appeared late in the Mesozoic era; its history was virtually confined to the Jurassic and Cretaceous times. Alhough known history is shorter it was much more varied, and the range of adaptation in these dinosaurs was wider than in saurischians (Fig. 2)

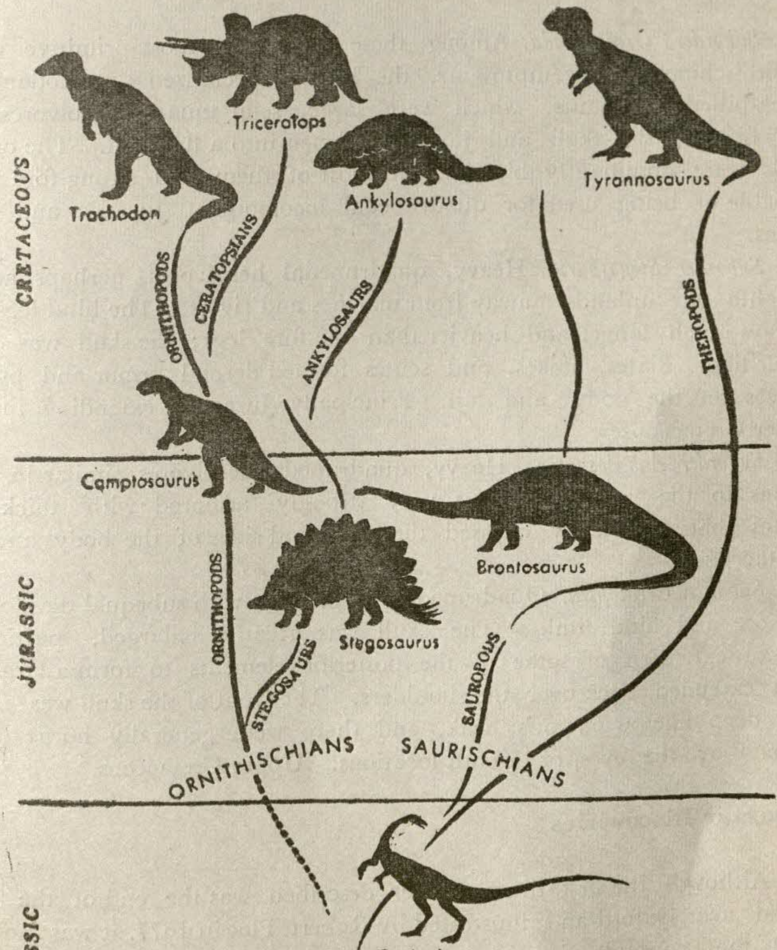


Fig. 2. Evolution of dinosaurs and their range in geological periobs (from Coldert 1962).

Outline Classification of Dinosaurs

ORDER SAURISCHIA

Suborder Theropoda. The first known dinosaurs, and the one group of persistent carnivorous types. These dinosaurs retained the bipedal mode of locomotion through their evolutionary history. Upper Triassic through Cretaceous.

Suborder Sauropoda. Giant, semi-aquatic, herbivorous saurischians, secondarily quadrupedal. The largest of the dinosaurs. Jurassic and Cretaceous.

ORDER ORNITHISCHIA

Suborder Ornithopoda. Among these were the most primitive of the ornithischians, the camptosaurs, the highly specialized trachodonts and duck-billed dinosaurs, which were large, semi-aquatic herbivores, with the front of the skull and jaws broadened into a flat bill. The ornithopods were dominantly bipedal, but most of them had strong fore limbs, capable of being used for quadrupedal locomotion. Jurassic and Cretaceous.

Suborder Stegosauria. Heavy, quadrupedal herbivores, perhaps adapted for life on 'uplands' away from marshes and rivers. The hind legs were always much longer and heavier than the fore legs; the skull was camptosaur-like. Plates, spikes, and scutes formed dermal armor and protuberances on the body and tail. Principally Jurassic, extending into the lower Cretaceous.

Suborder Ankylosauria. Heavy, quadrupedal herbivores, similar in adaptations to the stegosaurs, but very strongly armored with thick bony plates that completely encased the back and sides of the body and tail. Cretaceous.

Suborder Ceratopsia. Quadrupedal herbivores, with subequal development of fore and hind limbs. The skull was greatly enlarged, particularly by an extension of some of the posterior elements to form a large frill that extended back over the shoulders. The front of the skull was narrow and deep like a parrot's beak, and there were generally horns on the nose. above the eyes, or in both locations. Upper Cretaceous.

Dinosaur Discoveries

Although the first dinosaur ever described was the end of the femur found near Oxford and illustrated by Robert Plot in 1677, it was not until 1841 that concept of dinosaur was actually introduced. Richard Owen in his report on British Fossil Reptiles presented to the meeting of the British Association for the Advancement of Science in that year introduced the word 'Dinosauria'. He showed that dinosaurs were not just scaled up versions of other fossil reptiles but his studies on vertebrae and limb-bones established beyond any shadow of doubt that these animals held their limbs beneath their bodies in exactly the same way as mammals. Owen also produced restorations of *Iguanodon* and *Megalosaurus*. In 1878, miners in Bernissart, Belgium, while following a coal seam suddenly ran into sands containing masses of bones of over 30 complete skeletons of *Iguanodon*. Eleven of these were eventually mounted and are on display in the Royal Museum of Natural History, Brussels.

During the last three decades of the 19th century, world's greatest collections of dinosaurs were amassed as a result of the tremendous exertions of two dedicated fossil hunters in North America; Edward Drinker Cope and Othniel Charles Marsh. Unfortunately, they were deeply antagonistic towards one another. Each was determined to be the first in discovering and naming new types of dinosaurs. Their ruthless drive for bones and obvious hatred resulted in the largest collections of dinosaur bones ever made. Marsh was slow and painstaking in his work in contrast to brilliant and prolific Cope who published over 1400 papers, books and monographs. Both men had independent means and their entire fortunes were spent on financing and collecting dinosaur bones.

Since the beginning of this century, several major institutions with the financial backing of Andrew Carnegie have excavated complete skeletons of *Dipodocus* in Norith America. German expeditions, funded by public, made collections in East Africa during 1909–1912 which resulted in the discovery and mounting of *Brachiosaurs* in Berlin. Central Asiatic Expedition of the American Museum of Natural History in 1920, costing upto million dollars, explored for Mongolian dinosaurs. During last 40 years active exploration work is going on in most parts of the world especially in Poland, Russia, China, India, Thailand, Tanzania, Argentina, Australia and South Africa, in addition to Europe and the United States of America. Except for Antarctica, there is hardly any continent where dinosaurs have not been found. Whereas it would require enormous space even to summarise the sites of discoveries in various parts of the world (Fig. 3), it may suffice here to refer to some recent books and long articles which give an account of them. Colbert (1962, '68 '70 & '80), Ostrom (1979), Charig (1979), Halstead and Halstead (1981), Lambert(1983) have given accounts of the history, discovery and restorations of dinosaurs in detail.

Dinosaurs in India

In India, significant preliminary work has been done by British geologists during late 19th century; Hughes (1877) and Lydekker (1879) described some fragmentary bones of *Titanosaurus* from Central India and *Megalosaurus* from South India; Lydekker (1890) described an isolated

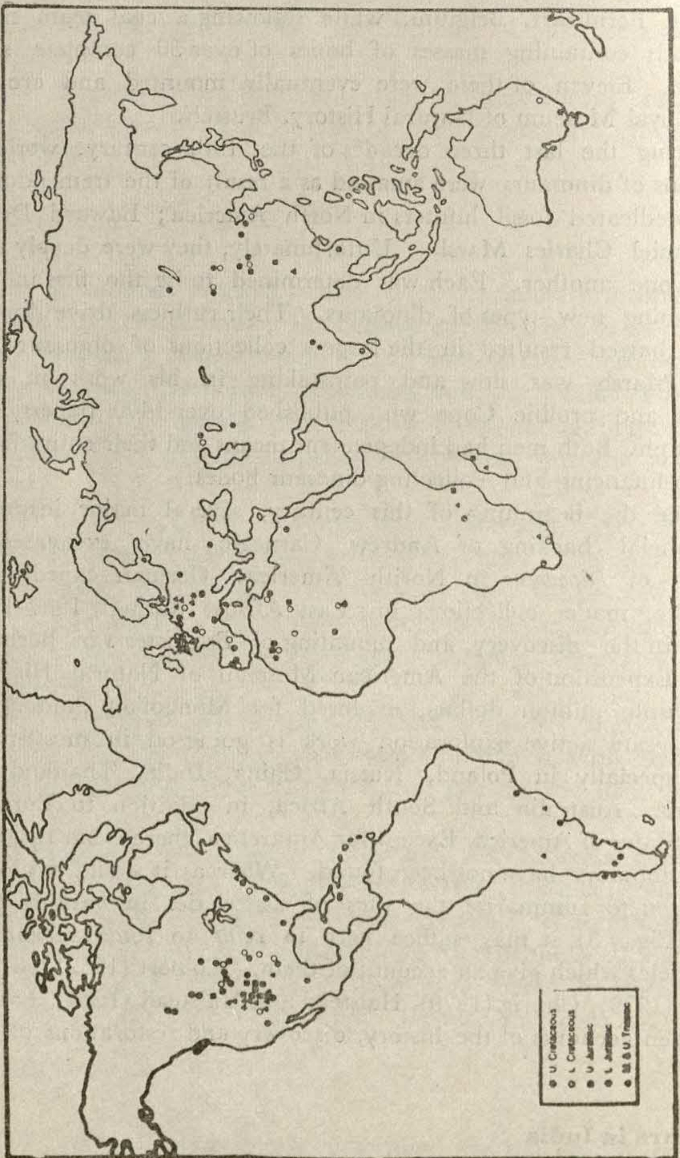


Fig. 3. A world map showing sites where dinosaur skeletal remains have been found (from Charig, 1979).

tooth of a theropod *Massospondyhus* from a place near Nagpur. The first large collection and study of dinosaurs was made during 1917–33 and described by Huene and Matley (1933) in a monograph. These Cretaceous dinosaurs included a number of Sauropod and Theropod genera and has been largely based on dissociated and isolated skeletal elements. The fossils were collected from Lameta Beds at Jabalpur, Chanda Dist., Nagpur Dist., Saugar Dist., and Tiruchirapalli Dist. Large sauropods include species of *Iitanosaurs*, *Laplatosaurus* and *Antarctosaurus*. The theropods include species of *Indosuchus*, *Massospondylus*, *Lametosaurus*, *Campsosuchus*, *Jubulpuria*, *Laevisuchus*, *Coeluroides*, *Ornithomimoides*, *Megalosaurus* etc. An armoured ornithopod, *Brachypodosaurus gravis* (Chakravarthi 1934), has also been reported from Jabalpur beds. An interesting feature of these dinosaurs is the resemblance to Madagascar dinosaurs of similar age. This suggests the possibility of a probable land bridge between India and Madagascar during Cretaceous times.

The evolution of dinosaurs during the Lower Jurassic times was at a particularly interesting stage when the two orders of the dinosaurs must have deployed adaptive radiations which have resulted in excellent faunas in Upper Jurassic and Cretaceous rocks. Yet our knowledge of dinosaurs of this period is extremely poor and because of this gap the knowledge of early dinosaur evolution is not well understood (Table I). An exciting find was made during early sixties (Jain, Robinson and Roychowdhury 1962) leading to the discovery of dinosaurs bones of Lower Jurassic period in Central India. A part of the dinosaur graveyard excavated in 1960–61 yielded about 10 tonnes of fossil bones which indicated presence

Table-I Distribution of Sauropod Genera and Species (in brackts) in Time and Space.

Age	Total	South America	North America	Europe	Asia	Africa	Australia
Upper Cretaceous	12 (19)	6 (2)	2 (2)	4 (4)	3 (4)	—	—
Lower Cretaceovs	11 (30)	—	3 (11)	5 (11)	2 (2)	3 (4)	1 (1)
Upper Jurassic	23 (46)	—	7 (10)	7 (10)	4 (5)	4 (8)	—
Middle Jurassic	4 (8)	1 (1)	2 (4)	2 (4)	—	2 (2)	1 (1)
Lower Jurassic	2 (2)	—	—	—	1 (1)	1 (1)	—

(*Source*: Jain *et. al.* 1979)

of several sauropod dinosaurs of different sizes. Subsequently, further excavations were carried out (Fig. 4) and a new sauropod dinosaur was identified as *Barapasaurus tagorei* (Jain, Kutty, Roychowdhury and Chatterjee 1975). To date, this is the only sauropod dinosaur of this geological period and is known by a large number of associated and dissociated bones (Fig. 5). A mounted skeleton of this dinosaur was exhibited for the first time in India at the Geology Museum, Indian Statistical Institute, Calcutta to the delegates of the 4th International Gondwana Symposium held in Calcutta in January 1977. Further results of analysis of characteristics of this dinosaur have shown that it is unique in some skeletal features (Jain, Kutty, Roychowdhury and Chatterjee, 1979), and therefore, must be placed in a new Family Barapasauridae (Halstead and Halstead 1981).

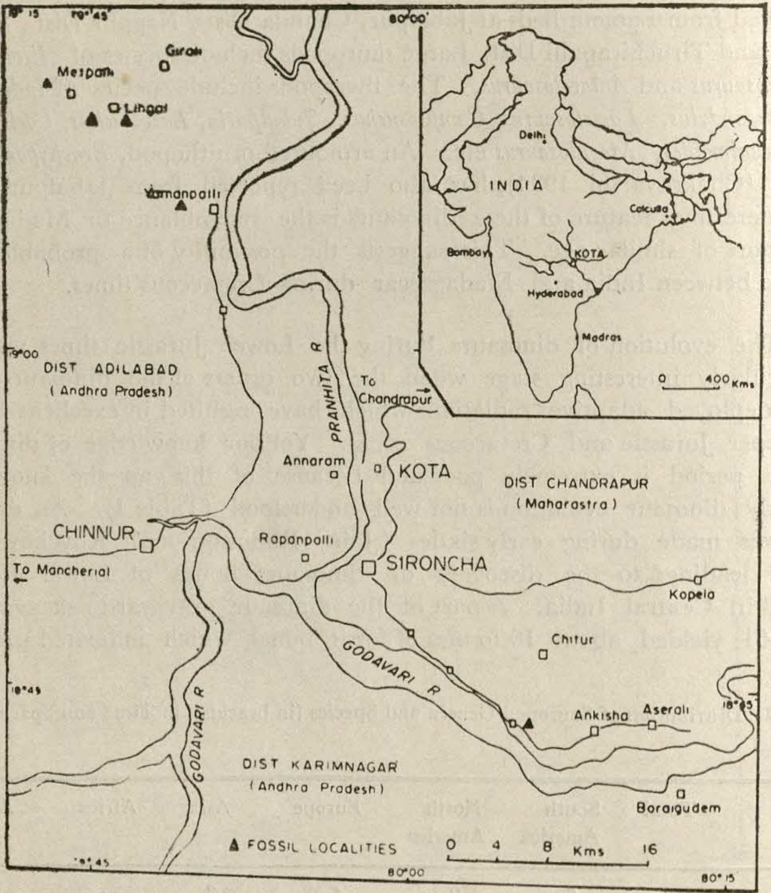


Fig. 4. Map of a part of the Godavari valley showing some sites where skeletal remains of *Barapasaurus tagorei* have been found (from Jain *et al*, 1975).

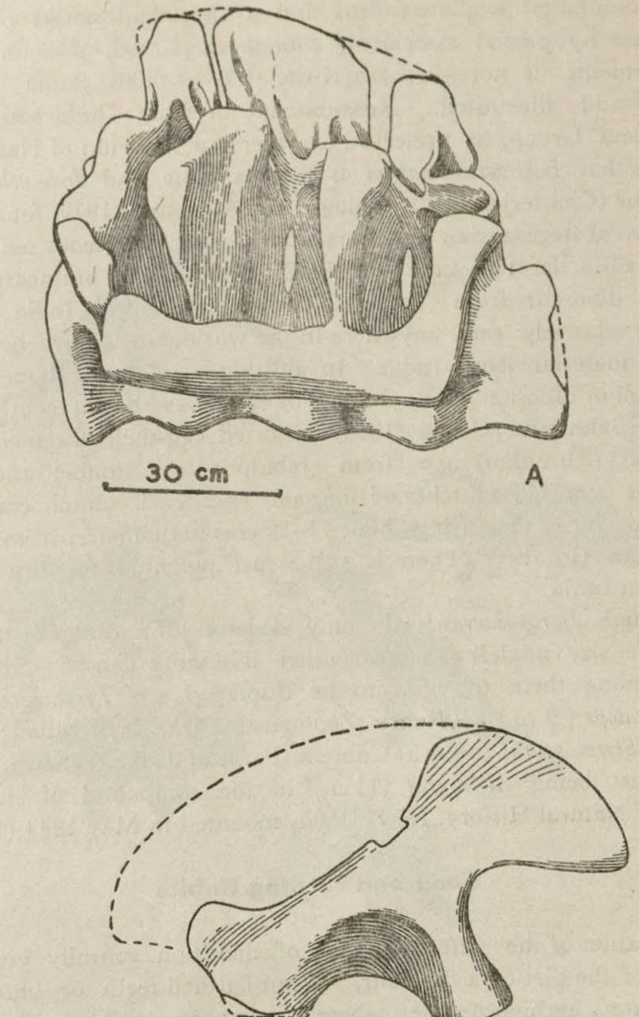


Fig. 5. The sacrum (above) and pelvis (below) of *Barapasaurus targorei* (from Jain *et al*, 1975).

Among the recent dinosaur discoveries from India, the finding of Upper Triassic dinosaurs (a plateosaurid and a thecodontosaurid prosauropod), represented by partial associated, though disjointed, skeleton and other bony elements, is noteworthy (Kutty 1969). This fauna is yet to be described and illustrated. Reassessment of Late Cretaceous carnosaurs from Lameta Group, as preserved in American Museum of Natural History has shown that *Indosaurus matleyi* is a megalosaur and *Indosuchus raptorius* a tyrannosaur (Chatterjee 1978). Yadagiri and Ayyasami (1979) found fragmentary remains of stegosaurian dinosaurs from Upper Cretaceous sediments near Trichurapalli. Berman and Jain (1982) described a braincase of a small sauropod dinosaur from Lameta Group in Central India. Brain-case fossils are relatively rare anywhere in the world, the above being the only preserved material from India. In addition to dinosaur bones, fragments of eggs shell of dinosaurs and clutches of eggs have been recently discovered in India. Sahni and Gupta (1982) reported egg-shell fragments of Upper Cretaceous (Turonian) age from Jabalpur sandstones; and Mohabey (1984) has reported clutches of dinosaur eggs, each clutch containing 3–7 eggs, and each egg measuring about 11–15 cms in diameter, from Cretaceous sediments in Gujarat. There is still a vast potential for future work on dinosaurs in India.

Although *Barapasaurus* is the only skeleton of a dinosaur in India on display, life size models of several other dinosaurs can be seen in several cities. Among them the first to be displayed are *Tyrannosaurs* (12 m) and *Triceratops* (9 m) at Nehru Zoological Park, Hyderabad; *Stegosaurus* (9 m) and *Styracosaurus* (5 m) at Cubbon Park, and Banerghatta, Bangalore and the last being *Allosaurus* (11 m) in the compound of the National Museum of Natural History, New Delhi, mounted in May 1983 (Fig. 6).

Food and Feeding Habits

An examination of the different types of dentition genrally gives a clear indication of the diet of a dinosaur. Worn flattned teeth or blunt peg-like teeth indicate a herbivorurs diet, whereas sharp serrated blade-like teeth are equally indicative of flesh-eating. In some dinosaurs there seem to have been large smooth stones (called *gastroliths*) in the stomach, just as in modern crocodiles. The crocodile, mainly an aquatic animal, swallows them deliberately and uses them not only to grind up its food into smaller pieces but also to adjust buoyancy in water.

In-Situ Discoveries

In a dramatic discovery of the tail of a herbivorous dinosaur, *Apatosaurus*, direct evidence has been found of scavenging habit of a carnivorous dinosaur, *Allosaurus*. The processes of the tail vertebrae were broken off and the bones themselves were found scared as if by knives. The spacing of these breaks and the scoring was exactly the spacing of the teeth of *Allosaurus*. Moreover a number of broken *Allosaurus* teeth have been found alongside this same tail. The fact that *Allosaurus* had bothered to feed on *Apatosaurus* tail is a strong evidence that it must be scavenging.

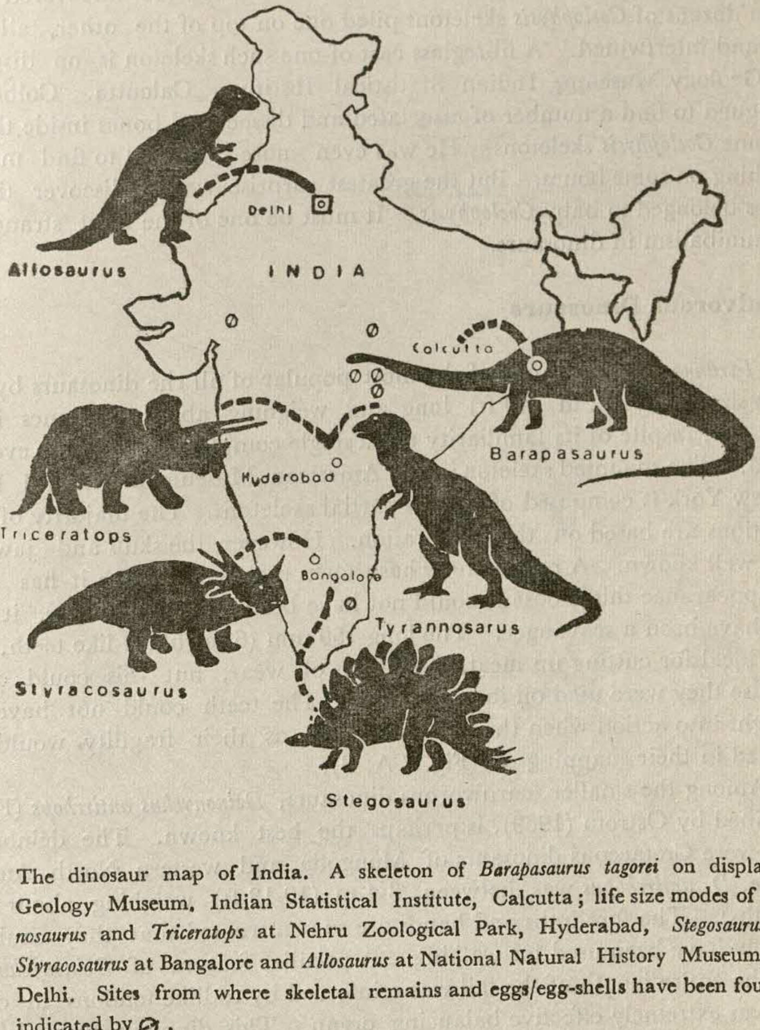


Fig. 6. The dinosaur map of India. A skeleton of *Barapasaurus tagorei* on display at Geology Museum, Indian Statistical Institute, Calcutta ; life size modes of *Tyrannosaurus* and *Triceratops* at Nehru Zoological Park, Hyderabad, *Stegosaurus* and *Styracosaurus* at Bangalore and *Allosaurus* at National Natural History Museum, New Delhi. Sites from where skeletal remains and eggs/egg-shells have been found are indicated by ⊘ .

Compsognathus, a small dinosaur (60 cm) weighing 3-3.5 Kg. about the size of a hen, has been known from Lithographic Limestone of Southern Germany. This dinosaur has been known for a long time to have minute bones within the rib-cage and these have been the subject of much speculation. Ostrom (1978a) identified them positively as belonging to *Bavarisaurus macrodactylus*, a lizard with an exceptionally long tail like iguanas and gekkos.

Compsognathus must have been an exceptionally fast and accurate hunter to catch and devour these lizards.

Another dinosaur *Coelophysis*, reaching a maximum length of 3 m (though smaller specimens only 1 m are also known), has been known from Upper Triassic of North America. E. H. Colbert excavated the site in 1947 from where original fragments were found in 1881. He uncovered dozens upon dozens of *Coelophysis* skeletons piled one on top of the other, all complete and intertwined. A fibreglass cast of one such skeleton is on display in the Geology Museum, Indian Statistical Institute, Calcutta. Colbert was intrigued to find a number of associated and dissociated bones inside the body of some *Coelophysis* skeletons. He was even more surprized to find marks of crushing on some bones. But the greatest surprise was to discover that the bones belonged to baby *Coelophysis*. It must be one of the most strange case of cannibalism in dinosaurs.

Carnivorous Dinosaurs

Tyrannosourus rex is one of the most popular of all the dinosaurs by virtue of its size, some 12 m (40 ft) long and weighing about 6 tonnes in life. However, inspite of its familiarity not a single complete skeleton has ever been found. The mounted skeleton in the American Museum of Natural History in New York is composed of several partial skeletons. The majority of reconstructions are based on this restoration. However, the skull and jaws have been well known. A recent study has shown that even though it has terrifying appearance this dinosaur could not have been an active hunter, it could only have been a scavanger. The long 150 mm (6 in) blade-like teeth, which were ideal for cutting up meat, show signs of wear, but this could only be because they were used on inert material. The teeth could not have been brought into action when the prey was alive as their fragility would have resulted in their snapping off (Fig. 7 A, B).

Among the smaller carnivorous dinosaurs, *Deinonychus antirrhops* (Fig. 8), described by Ostrom (1969), is perhaps the best known. The deinonychosaurs were Cretaceous dinosaurs of Mongolia and western North America. In life, *D. antirrhopus* was between 3-4 m (10-13 ft) weighing about 68 kg (150 lbs). The forelimbs and hands are so constructed that they could not possibly have been used for walking. A peculiar feature of *Deinonychus* was that the entire length of the tail was reinforced by rodlike tendons in ossified state–an extremely effective balancing organ. This dinosaur was also provided with huge, sickle-like bones (about 10 cms) on one toe of each foot. In life, sharp, curved, nail like sheaths covered these claw bones. They served as weapons to kill prey. When not in use, these claws were carried in a retracted position, so as not to be damaged.

Although pack hunting is fairly common is carnivorous mammals, evidence indicates that *Deinonychus* probably hunted in packs. At a site in U.S.A., together several specimens of *Deinonychus* a few parts of a single herbivorous dinosaur, *Tenontosaurus* have been found. The latter weighs around 450 Kg. Although the more common item of *Deinonychus* was probably small fast moving prey but in packs they were capable of attacking prey six-times their sizes.

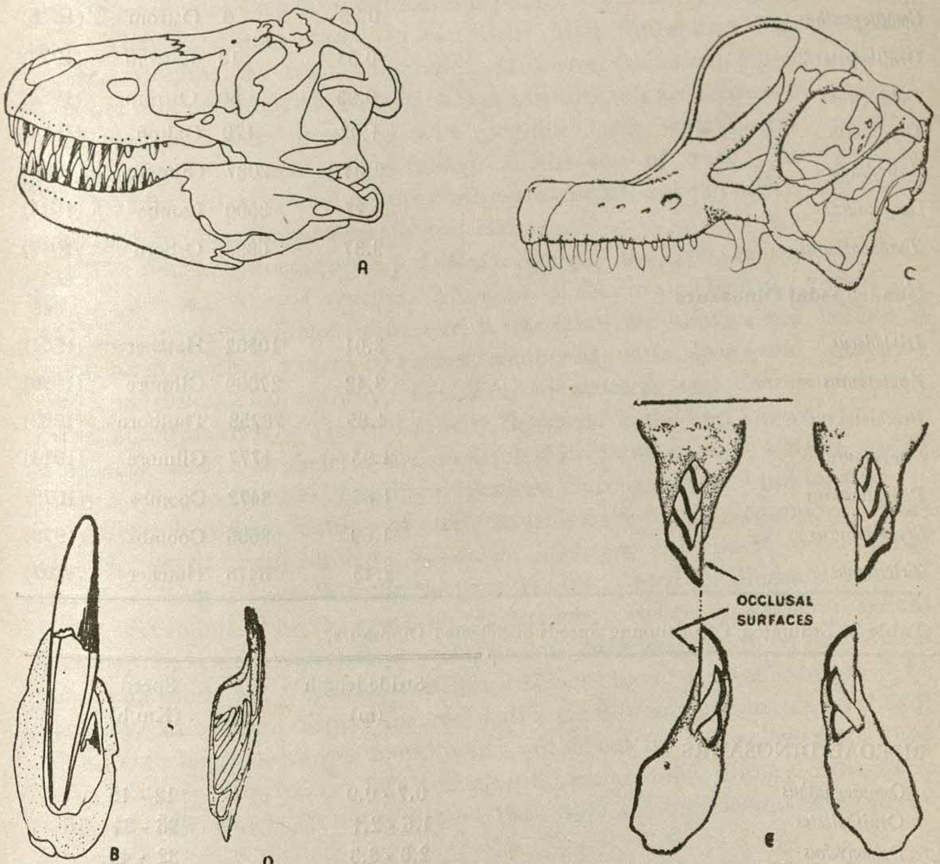


Fig. 7. A life restoration of a specialized carnivorous dinosaur, *Deinonychus antirrhops* from Lower Cretaceous of Montana, U.S.A. (from Ostram 1969).

Sauropod Dinosaurs

The sauropads, popularly known as brontosaurs may be called the very essense of what is recognised as a dinasaur. Traditional view of these dinosaur is that they were slow, clumsy, ponderous animals. However, recent studies by Thulborn (1982, Table II and III) show that walking, trotting and runnig speed of some souropods may be 2.4, 8.8 and 20.4 Km/h, respectively. Their habitiat has been the subject of much speculation and debate

Table—2. Height and Weight of Selected Dinosaurs.

	Height (m)	Weight (kg)	Source
Bipedal Dinosaurs			
Compsognathus	0.23	3	Ostrom (1978)
Ornitholestes	0.53	18	Ostrom (1978)
Deinonychus	0.95	68	Ostrom (1969)
Dryosaurus	1.02	120	Galton (1974)
Antrodemus	2.04	2087	Gilmore (1920)
Gorgosaurus	2.77	3500	Lambe (1917)
Tyrannosaurus	3.37	6895	Osborn (1917)
Quadrupedal Dinosaurs			
Diplodocus	3.01	10562	Hatcher (1901)
Apatosaurus	3.42	27869	Gilmore (1936)
Brachiosaurus	4.85	78258	Thulborn (1982)
Steogosaurus	1.95	1777	Gilmore (1914)
Panoplosaurus	1.66	3472	Coombs (1978)
Styracosaurus	1.69	3686	Coombs (1978)
Triceratops	2.43	8478	Hatcher (1907)

Table-3. Estimated Top Running Speeds of Selected Dinosaurs.

	Stride length (m)	Speed (Km/h)
BIPEDAL DINOSAURS		
Compsognathus	0.7 - 0.9	12 - 15
Ornitholestes	1.6 - 2.1	26 - 34
Deinonychus	2.8 - 3.8	32 - 42
Dryosaurus	3.0 - 4.1	33 - 43
Antrodemus	4.1 - 5.0	13 - 18
Albertosaurus	5.5 - 6.8	15 - 21
Tyrannosaurus	6.7 8.3	16 - 23
QUADRUPEDAL DINOSAURS		
Diplodocus	3.2	11.5
Apatosaurus	3.4	12.3
Brachiosaurus	4.9	17.6
Stegosaurus	2.0	7.0
Panoplosaurus	2.2	7.8
Styracosaurus	6.1	22.1
Triceratops	7.3	26.4

(*Source* - Thulborn R. A. 1982)

for a long time, and the main arguments have recently been summarized by Coombs (1975). Although the sauropods were herbivores, direct evidence of diet has not been forthcoming in most cases. Long necks could have been used for browsing on trees, or along stream-banks or under water. Axial and appendicular skeletal modifications point to primarily terrestrial behaviour. They sometimes reared up on their hind limbs and they definitely entered streams, at least sometime. However, sedimentological evidence does not support immersion in deep lakes as sauropods are frequently pictured. These dinosaurs were provided with peg-like teeth which seem singularly inappropriate for feeding on foliage at the top of trees, like giraffes. (Fig 7 c D). Most probably, the teeth were used to rake in soft plant material along the margins of swamps and lakes.

The best known sauropod is *Diplodus carnegii*, the type skeleton of which is mounted at the Carnegie Museum of Natural History, Pittsburgh. Although it was the longest dinosaur, it was relativeiy slim, since in life it might have weighed a mere 10 tonnes, compared with *Apatosaurus* weighing nearly 3 times. Collectively, *Diplodocus Apatosaurus* and *Dicraeosaurus* are called atlantosaurids. The reason why *Diplodocus* is the best known dinosaur is because casts of its skeleton are on display in most of the world's major muesums due to financial backing of Andrew Carnegie, a steel millionaire.

Although skeletal features of atlantosaurids have much in common, important features distinguish them. However, *Apatosaurus* is based on a fairly complete skeleton; and later *Brontosaurus* was introduced for dinosaur material which was found to be the same as *Apatosaurus*. Hence, *Brontosaurus* should properly be called *Apatosaurus*. *Apatosaurus* skeleton, mounted in several American musuems, was discovered in 1979 to have a wrong head. The skeleton had been on display for over half a century with abbreviated head, till a much longer skull was found which got mixed in the collections. The error has been detected recently and most museums now display *Apatosaurus* with a skull which is more robust than that of *Diplodocus*, but equaliy elongated.

Table-4. Estimated Speeds of Selected Dinosaur Groups in walking Trotting and Running.

	WALK Km/h	TROT Km/h	RUN Km/h
Coelurosaurs	2.2	7.8	18.2
Small ornithopods	1.7	6.1	14.2
Carnosaurs	3.5	12.7	29.6
Larger ornithopods	3.2	11.4	26.2
Prosauropods	2.4	8.8	20.4
Sauropods	4.8	17.2	40.0
Stegosaurs	3.5	12.6	29.4
Ankylosaurs	3.0	10.9	25.5
Ceratopsians	2.8	10.2	29.7

(*Source* - Thulborn, R. A. 1982)

Hadrosaurid Dinosaurs.

The hadrosaurs or duck-billed dinosaurs represent the peak of the evolution of the bipedal herbivores. Their tails were more flattened from side to side than those in other dinosaurs. The feet had three toes which bore hooves, the first and fifth toes having been lost. The feature which gives these dinosaurs their popular name is tbe flattening of the front of the snout and its expansion to from a duck-like bill. The edge of the predentary bone at the front of the lower jaw had rough crenulations, and this was used to chop plants against the upper duck bill.

The dentition shows important specializations. There were several longitudinal rows of teeth in each jaw, between 45 and 60 vertical rows each with about 6 successional teeth one above the other, giving a total of between 1600 and 2000 teeth in the jaws. In the lower jaw, there were three longtudinal rows and in the upper two. The inner margin of the lower teeth and the outer of upper had a layer of highly mineralised enamel. As the two tessellated pavements of the wear surfaces dragged across each other during chewing, the differential hardness of the enamel and the softer dentine ensured the mantenance of sharp ridges which served to grind up tough plant material, in exactly the same way that horses and cows grind up their food.

The development of muscular checks helped in the general chewing process. The hadrosaurs represent one of the few herbivorous dinosaurs which provide direct evidence of their diet. In number of specimens there are plant remains preserved in their stomachs and these consist of pine needles, twigs and pinecones. This confirms the view derived from a study of the teeth and jaw apparatus that the hadrosaurs were capable of coping with exceedingly tough plant material.

Ceratopsian Dinosaurs

The ceratopsians or horned dinosaurs represent a major group of Upper Cretaceous Ornithischian dinosaurs. Typically this group is characterised by the development of an enormous bony frill from the posterior part of the skull which extended over the neck region. In some cases the posterior margin of the frill was drawn out into long spikes and in the majority of forms there were median horns on the nasal region of the snout as well as paired horns above and behind the eyes.

The facial part of the skull was compressed in front from side to side to form a sharp beak, some what like the beak of a turtle, or more exactly like the beak of a parrot grown to giant proportions. The front of the lower jaw likewise was in the form of a pointed beak. There were no teeth in this beak, but in the sides of the jaws were complex batteries of cheek-teeth that served to chop and slice vegetation upon which the dinosaurs fed. Each tooth is in the form of a vertical rhomb that spliced past its opposing tooth with a scissor-like action, and it terminated at its base in two diverging roots, which enclose the crown of tooth soon to erupt as the worn tooth drops out There is vertical row of replacing teeth above each functioning tooth in the skull, and below each functioning tooth in the lower jaw (Fig. 8E). There is, therefore, a constant succession of new teeth, maintaining a never-ending supply. There is a long row of functioning teeth on each side of the skull and of the lower jaw, often as may as 36 to 40 in a large ceratopsian. Therefore, there may be as many as 160 functional teeth and 480 teeth waiting to come up to use. As such, it may be concluded that at a time ceratopsians were provided with a formidable dental battery containing as many as 640 teeth.

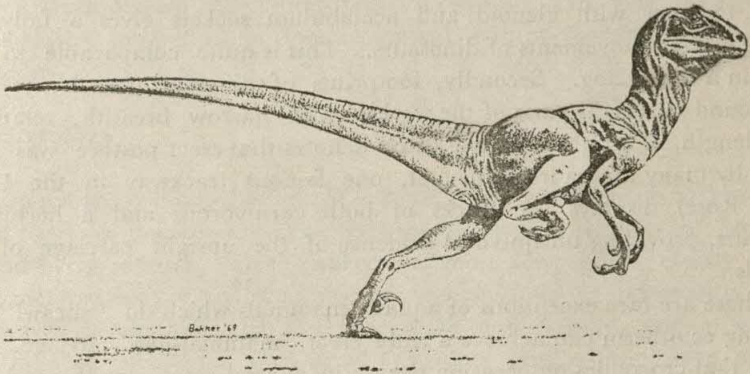


Fig. 8. Skull and jaws of *Tyrannosaurus rex* (A) and section of lower jaw to show replacing teeth (B) ; skull of *Brachiosaurus brancai*, a herbivorous sauropod (C) and section of upper jaw of *Diplodorus longus* to show replacing teeth (D) : E, Occlusal surfaces of upper and lower jaw (in section) of *Triceratops* (from various sources).

Naturally, the question would arise about the food of ceratopsians which required such an elaborate mechanism for cutting and shearing plant food. It has been suggested that such a profusion of teeth, constantly having replaced, indicate that these dinosaurs must have fed upon tough, fibrous plants, such as cycads and palms. *Pentaceratops* fossils discovered in New Mexico have been found associated with sabalites palms, cycads and araucarian conifers, all containing tough fibres. In addition, several speeies of figs and grapes, as well as viburnum, willows, poplars and other broad-leaved trees have also been found. It seems, therefore, that the ceratopsians had a wide variety of choice among the vegetation around and probably could pick and choose.

Dinosaur Physiology

An area of understanding about dinosaurs which is actively receiving the attention of world-wide palaeontologists is related to their thermal regulation.

Although sharp difference of opinion exists among some, the main arguments centre around the following points :

Posture and Gait

Present day reptiles such as crocodiles, tortoises, lizards, *etc.*, have a sprawling posture and low profile. In contrast, most extant mammals have an erect posture and high profile. Even birds have an erect posture and a profile similar to mammals. It has been suggested that sprawlers are ectotherms and erect posture and gait occurs only in endotherms.

Evidence for erect posture of dinosaurs is adduced on two counts. Firstly, the shape of forelimb and hind limb bones, specially humerus and femur, together with glenoid and acetabulum sockets gives a faily good picture of limb-movements of dinosaurs. This is quite comparable to those bones in a cat or dog. Secondly, footprints of dinosaur (trackways) have been found in several parts of the world. The narrow breadth, relative to stride length, of all dinosaur trackways denotes that erect posture was maintained by many dinosaurs. In fact, one famous trackway in the U.S.A. (Glen Rose) displays the tracks of both carnivorous and a herbivorous dinosaurs, providing unequivocal evidence of the upright carriage of both animals.

There are rare exceptions of aquatic mammals which do "sprawl", but no living ectotherm can achieve a truly erect, mammal-like carriage. It is known that crocodiles on occasion rise up in a semi-erect stance, and some species of lizards (*Chamaeleo*) achieve a somewhat bow legged upright stance. These are, however, rare and distinctly non-mammal-like exceptions among reptilian locomotory adaptions.

Critics of this hypothesis have pointed out that no cause-and-effect relationship between posture and physiology has been established. It appears that ectotherm metabolism and temperature regulation impose major physiological obstacle which makes erect posture and locomotion impossible. Surely, it is no coincidence that nearly all living animals with erect posture are endotherms.

Growth Rings on Dinosaur Teeth

An altogether different line of argument has been put forth on the basis of dinosaur teeth. Transverse sections of teeth of tyrannosaurid (carnivorous) dinosaur have been examined by P. A. Johnson (1979). These reveal rings which can be distinguished into a narrow band followed by a broad band. As many as eight alternating bands have been reported. It has been proposed that, like similar bands in other skeletal tissues of ectoterms, these indicate growth rings. Such growth rings have been known in trees and fish scales for a long time. Fossil crocodiles have also been found to have similar growth rings. It has been suggested that the dinosaur physiology (ectothermic) did not permit high level of activity during cold season as compared temperatures and hence the presence of alternating narrow and to warm broad bands of growth.

Johnson's article immediately received a number of counter arguments (*Nature*, Vol. 288, 13 November 1980). Among various objections raised was the well documented evidence of active replacement of teeth in all dinosaur groups. This goes on throughout their life. Crocodiles are also known to shed teeth once in two years but old crocodiles may become toothless when no more replacement takes place. A life span of eight years (as implied by eight alternating rings) for a dinosaur tooth appears untenable. In addition, distinct annulations have also been recorded from the teeth of large mammal such as African buffalo and desert bighorn sheep. The ring may rather indicate seasonal variation in food resources and not seasonal temperature fluctuations.

Bone Histology

Through the combined efforts of a large number of scientists all over the world it has been possible to learn about the histological features of bones of fossil and living reptiles. To the naked eye, most long bones clearly show two distinct patterns of organisations : a compact area of densebony tissue which is well developed in shafts and forms the outer region, and a spongy area of porous tissue which often forms the inner part of the bone and is well developed near epiphyses. Intermediate conditions between compact and spongy tissue are found in various regions and circumstances. In the long bone shafts, the compact tissue is traversed by primary vascular canals which have charcteristic structures. These are formed during primary bone deposition and can be seen in common living ectotherms, e.g., frog, lizard, etc. In contrast, the campact bone of many mammals and birds commonly contain large number of vascular channels which are secondary in origin. A mature compact bone tissue having these vascular channels gives rise to the *Haversian system* whicn is a common feature in birds and mammals. The occurance of Haversian in birds and mammals has been attributed to their high metabolic rate and endothermic physiology. It is interesting to note that dinosaur bone also exhibits a very similar vascularised Haversian construction and hence the implication that dinosaurs were endotherms (Fig. 9).

Critics of this hyphthesis have pointed out that some ectotherms (e. g., turtles and crocodilians) have a secondary Haversian system and the same is absent in many small endotherms, both in birds and mammals. In 1980, A. J. Ricqles, who has been studying bone histology for over a decade, suggested that Haversian system in large, adult dinosaur indicates long individual life spans comparable to large living land mammals and that their physiology was also somewhat similar. It is, therefore, suggested that at least large adult dinosaurs were warm blooded, unless an alternative explanation of Haversian system is available.

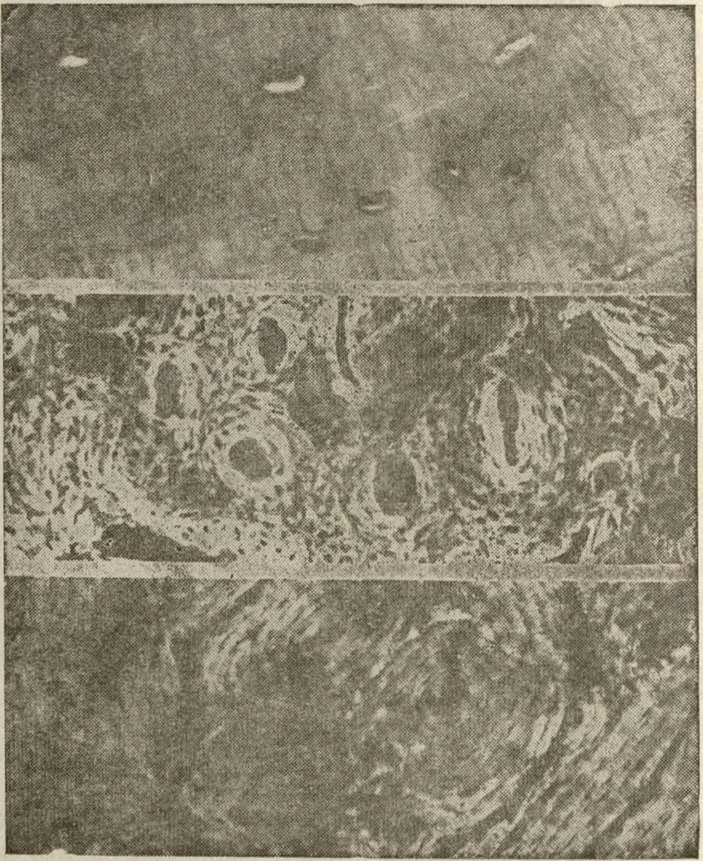
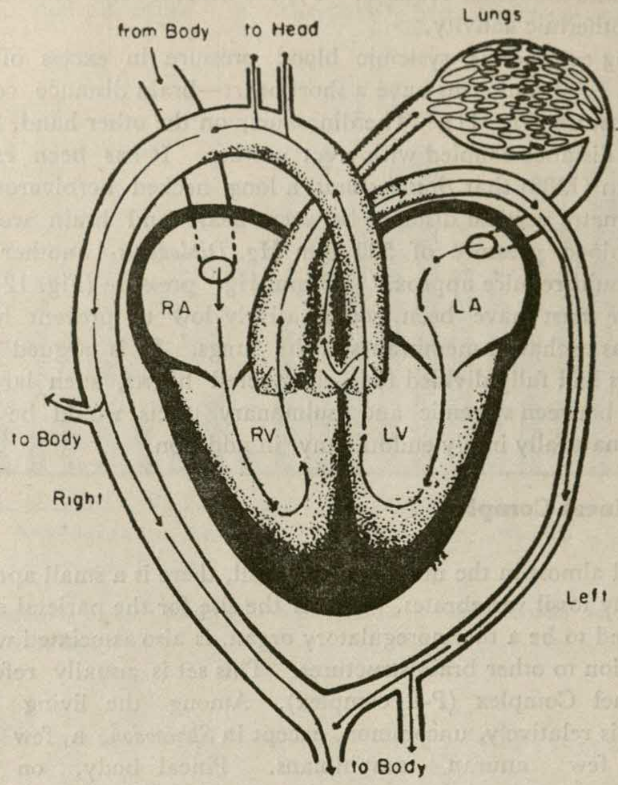


Fig. 9. Bone histology of some vertebrates. *Above*, section of a lizard bone; *Middle*, section of a dinosaur bone and *below*, section of a mammal bone. Note the presence of *Haversian system* in dinosaur and mammal (from Charig, 1979).

Heart and Blood Pressure

Nobody has ever seen a dinosaur heart nor, for that matter, any soft tissue except on rare occasions when skin, flesh and such delicate organs as internal ear have been found preserved. Therefore, any discussion on dinosaur heart and blood pressure can be on hypothetical grounds only. There is no exact living counterpart of any dinosaur. Yet the discussion on endothermy leads, naturally, to the structure and functioning of the heart and its capability to supply blood to various parts of the body of an animal of vast proportions. Recenty, P. J. Regal and Carl Gans (1980) and J. H. Ostrom (1980) have made very interesting interpretation of dinosaur heart and blood pressure, respectively.



Eig. 10. Model of blood flow through "B" type heart of crocodilian (from Regal and Gans, 1980).

In reptiles, birds and mammals (amniotes), the heart is a double pump. The heart pumps blood to the lungs, from where blood does not pass to the body directly. Instead, it passes through the heart a second time and is only then delivered to the body tissues. Its efficiency depends on how well the two streams of blood (oxygenated and deoxygenated) are kept apart. In birds and mammals, separation is assured because of presence of septa to divide the two circulations. In reptiles there are two patterns: one exemplified by turtles, lizards, snake, etc. (Type A), and the other by crocodilian (Type B). There is considerable admixture of oxygenated and deoxygenated blood in Type and deoxy gnatedA heart. It has been suggested by Regal and Gans (1980) that dinosaurs probably did not have Type A heart. Studies on Type B heart in crocodiles have snggested that there is relatively complete seperation of systematic and pulmonary circulation under resting conditions. (Fig 10) However, it may not be efficient in separating oxygenated and deoxygenated blood during intense activity. It has been suggested that the cardiovascular arrangement in dinosaur was probably similar to that of crocodilians, which otherwise also share basic archosaur features. Type B heart is also capable of maintaining endothermic activity.

In living ectotherms, systemic blood pressure in excess of 80mm Hg is unknown. All ectoterms have a short heart—brain distance coupled with sprawling posture (Fig. 11). The dinosaurs, on the other hand, had a long heart-brain distance coupled with erect posture. It has been estimated by J. H. Ostrom (1980) that *Brachiosaurus*, a long necked herbivorous dinosaur having a 6 metre vertical distance between heart and brain would require a systemic blood pressure of 500 mm Hg. *Diplodocus*; another herbivorus dinosaur, would require approx. 450 mm Hg. pressure (Fig. 12). The systolic pressure must have been comparatively low to prevent haemorrhage across the gas exchange membranes of the lungs. It is argued that unless the dinosaurs had fully divided four-chambered hearts, such large pressure differantials between systemic and pulmonary tracts would be impossible. This would naturally imply endothermy, in addition.

Parietal-pineal Complex

Situated almost in the middle of the skull, there is a small aperture (foramen) in many fossil vertebrates. This is the site for the parietal eye. Pineal body, believed to be a thermoregulatory organ, is also associated with parietal eye in addition to other brain structures. This set is usually referred to as Parietal Pinel Complex (P-P complex). Among the living vertebrates, parietal eye is relatively, uncommon, except in *Sphenodon*, a few families of lizards and few anuran amphibians. Pineal body, on the other hand is found in all quadrupeds except edentates, dugongs and crocodilians.

P–P complex is commonly present among fossil reptiles, except in many mammal-like reptiles and all dinosaurs (Fig. 13). Experimental work on some lizards by removal of P–P complex has suggested that temperature regulation is a major function of this complex. It has been suggested that partial loss of P–P complex or its absence among fossil reptiles provides an indication of thermoregulatory mechanisms. As such, the mammal-like reptiles and dinosaurs lacking P–P complex may have been endotherms like mammals.

Contrary arguments have also been forwarded. Firstly, P–P complex is absent in crocodiles as well, both living and fossil. Therefore, its absence may be an ancestral character of Archosauria, not necessarily related to endothermy. Secondly, it has been argued that loss of P–P complex in dinosaurs could be in response to gradually warming climates of the Mesozoic. The dinosaurs, while still retaining ectotherm strategy, could effectively be controlling temperatures due to their large size. Some small dinosaurs retaining pineal may have been endothermic.

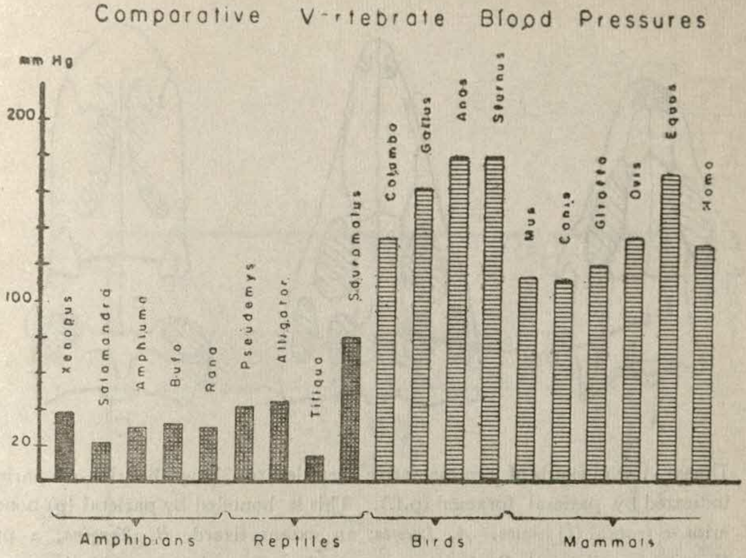


Fig. 11. Comparative blood pressures of some ecto and endothermic vertebrates (from Ostrom, 1980).

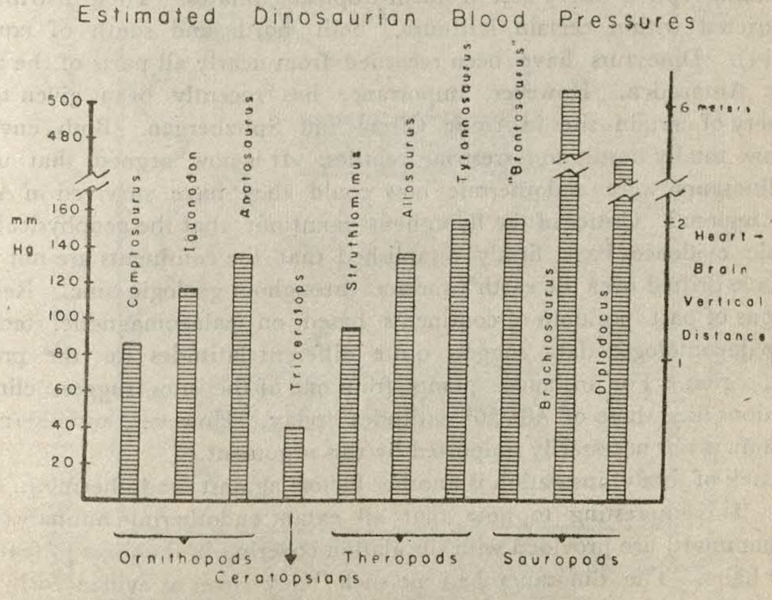


Fig. 12. Estimated dinosaur blood pressures (equivalent to hydrostatic pressure of fluid columns equal in length to the vertical distances between the heart and brain) as deduced from mounted skeleton (from Ostrom, 1980).

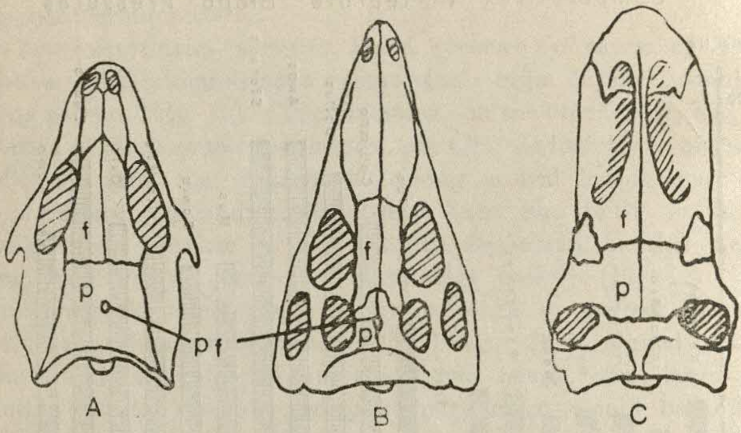


Fig. 13 Dorsal view of skulls of representative reptiles to show the site of parietal eye indicated by parietal foramen (p.f.). This is bounded by parietal (p) bones, posterior to frontal (f) bones. *A*, *Lacerta*, an extant lizard, *B*, *Yongina*, a primitive Permian reptile and *C*. *Camarasaurus*, a late Jurassic sauropod dinosaur. Note the presence of parietal foramen in A and B and absence in C. (from Jain, 1981).

Dinosaur Distributions and Body Insulation

Extant reptiles thrive best in warm tropical climates. Their distribution is restricted within certain latitudes, both north and south of equator (Fig. 14). Dinosaurs have been recorded from nearly all parts of the globe except Antarctica. However, importance has recently been given to the discovery of certain sites in Arctic Circle and Spitzbergen. Both environs are now totally hostile to terrestrial reptiles. It is now argued that unless the dinosaurs were endothermic how could they have survived in Arctic Circle regions? Critics of the hypothesis point out that the geophysical and tectonic evidence have firmly established that the continents are not fixed and have drifted over the earth's surface throughout geologic time. Reconstructions of past positions of continents based on palaeomagnetic, tectonic and palaeontologic data suggest quite different latitudes for the present Arctic region. For instauce, plants from one of the sites suggest climatic conditions like those of 40^0–50^0 latitudes today. However, endothermv in dinosaurs is not necessarily supported by this argument.

Lack of body insulation is another factor against endothermy in dinosaurs. It is interesting to note that all extant endothermic animals (birds and mammals) are provided with insulation covering in the form of feathers, fur or hairs. The dinosaurs had no such body cover as evidenced by preserved skin in several cases. It has been suggested that dinosaurs did not need an insulation due to prevailing warm climates. But the cooling of the earth at the end of Mesozoic hit the dinosaurs severely. Whereas several reptiles such as turtles and crocodiles were able to find cozy nooks and

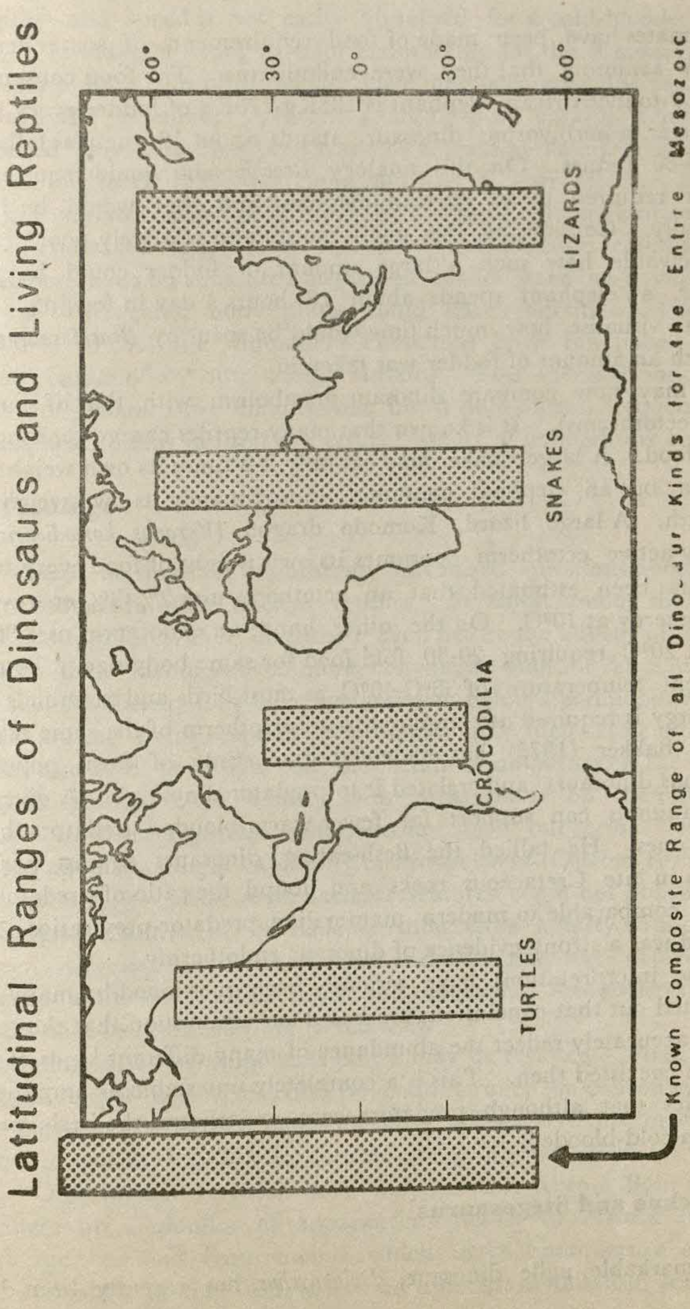


Fig. 14 Latitudinal ranges of dinosaurs and living reptiles (from Ostrom, 1980).

corners to survive the winter by hibernation, and small fur-covered mammals built warm nests, the dinosaurs could not do so. Most of them were too big to find protective caves or burrows to hide. They stayed out and died.

Dinosaur Food Requirements

Estimates have been made of food requirements of some herbivorous dinosaurs assuming that they were endotherms. The food consumption of an adult 7-tonne African elephant is 135Kg-270Kg of fodder every 24 hours. *Brachiosaurus*, a herbivorous dinosaur, stands about 12 metres tall and may weigh 70-80 tonnes. On thls analogy, *Brachiosaurus* would require 10-times of fodder required by an African elephant and that would be 1.3 to 2.7 tonnes daily. Considering the small mouth and relatively poor dentition it is inconceivable how such a large amount of fodder could be ingested. Mereover, an elephant spends about 18 hours a day in feeding. It is impossible to visualise how much time would be spent by *Brachiosaurus* in feeding if such an amount of fodder was taken in.

We may now compare dinosaur metabolism with that of some extant reptiles (ectotherms). It is known that many reptiles can go for long periods without food. A large snake may eat only 2-3 times its own weight over an entire year but an elephant consumes approximately its own weight in food in a month. A large lizard, Komodo dragon (*Varanus komodiensis*), which is a fairly active ectotherm consumes its own weight of food every two months. It has been estimated that an ectotherm uses 2%-3% energy at 20°C and 1% energy at 10°C. On the other hand, an endotherm uses 80%-90% energy at 20°C requiring 20-30 fold food for same body size. To maintain an ambient temperature of 38°C-40°C, as most birds and mammals do, 5-10 times energy is required as compared to an ectotherm of the same size.

R.T. Bakker (1975) has made elaborate study of fossil population of herbivorous dinosaurs and related it to predator dinosaurs. A given population of animals can support far fewer warm-blooded predators than cold-blooded ones. He tallied the flesh-eating dinosaurs against herbivorous dinosaurs in late Cretaceous rocks and found the ratio of predator to prey very low comparable to modern mammalian predator-prey ratios. This he concluded was a strong evidence of dinosaur endothermy.

These interpretations have seriously been questioned by many authors. It is pointed out that conclusions are based on assumption that the specimens collected accurately reflect the abundance of many different kinds of dinosaurs which coexisted then. This is a completely untestable assumption. This also implies that although predators were endotherms the herbivores could have been cold-blooded.

Deinonychus and Stegosaurus

A remarkable, agile dinosaur, *Deinonychus*, has recently been described and restored. Its skeletal specializations suggest that it could not walk or even stand on all four legs. It had strongly recurved talons on its feet. It is visualised as jumping from one foot to another and kicking out at its prey or attacker with its free foot. The talons used for slashing in this manner must have been used in close coordination and with a keen sense of balance. Such agility and speed is not easily visualised for a cold-blooded reptile. Ostrom (1978) makes a strong case for endothermy for this dinosaur.

The bony back fins of *Stegosaurus* have long been regarded as protective armour against predators. A recent studv of these curious bony plates of the armoured *Stegosaurus* has been wade by a team at Yale University which included an engineer (Farlow, Thompson and Rosner 1976). This study found that the plates were penetrated by a complex network of canals that must have contained large blood vessels. What function could they have served? Experiments on simulated *Stegosaurus* model have suggested that the double row of staggered bony plates could have functioned as convective heat-loss fins to cool the dinosaur. These may have functioned like the vanes and baffles of an automobile radiator. This investigation does not prove that *Stegosaurus* was endothermic but it does suggest frequent need to shed excess heat. This in itself is a critical aspect of temperature regulation.

Recent Findings

The debate on dinosaur endothermy has raised questions about possible warm-bloodedness in other ancient reptiles. Foremost among them are the mammal-like reptiles which flourished even before the advent of dinosaurs. Evidence in their favour is even more convincing than for dinosaurs. This implies that endothermy may have evolved even before the dinosaurs. Other contenders are the flying reptiles (Pterosaurs) and their close allies. The debate has also shed new light on the origin of dinosaurs on the one hand and on their possible close affinity with *Archaeopteryx* on the other. The latter has so far been regarded as the earliest bird but serious doubts have been raised about the flight capability of *Archaeopteryx* (Ostrom 1979). How can it be ancestral to birds if its skeletal features could not possibly permit flight? Several features in its skeleton show close affinity with dinosaurs. It has been suggested that *Archaeopteryx* and birds may have evolved from a small theropod dinosaur. Like birds and dinosaurs, *Archaeopteryx* was also possibly an endotherm, according to this view.

In conclusion, a few important points may be restated. First, although dinosaurs have not been proved to be endothermic, the collective evidence is highly suggestive. Second, there are large areas where our understanding is inadequate which has led to contradicting interpretation of structural features. Only further research work may possibly throw light on them. Third, there are a number of apparent anomalies in certain features of dinosaurs such as food requirements which make endothermy difficult to conceive. Could it be that dinosaurs had different metabolism and peculiarities of thermal physiology than any extant animal with which comparisons have been made? Whatever must have been the set-up, it cannot be denied that they survived for a pretty long time. It is a challenging task for the scientists to interpret the precise metabolism and physiology of dinosaurs on the basis of fossil bones alone.

Eggs, Embryos & Nestling Behaviour

Knowledge about dinosaur eggs and embryos is extremely scanty in sharp contrast to the abundance of dinosaur bones in some regions. Even when fossilized bits of egg-shell or even whole eggs are found it is often difficult to decide *which* dinosaur laid the eggs. Uncertainty may also arise whether the eggs belong to some other reptile or even a large bird. Inspite of such difficulties some remarkable discoveries have been made giving an insight into this aspect of dinosaur palaeobiology.

Earliest Discoveries

An expedition from the American Museum of Natural History to Mongolia in 1922 discovered a large and rich deposit of small dinosaur skeletons, together with numerous elongated fossil eggs. More than seventy eggs and countless thousands of fragments of egg-shells were found initially. Since then Russian and Chinese scientists have added to this record. Each egg was about 20 cm (8 in) in length with a shell the surface of which is roughened by many wrinkled ridges. In life, it was probably rather leathery, as is commonly the case among large modern reptiles. Many of the eggs were found in clusters-obviously nests. The skeletons were of an ancestral horned Cretaceous dinosaur, *Protoceratops*, and it was the definite *association* of these skeletons with fossil eggs that clinched the matter. There could be no doubt that these were the eggs of the dinosaurs.

So far over a hundred skeletons have been recovered from Mongolia. Many of these exhibit growth stages from hatchling to adult (Fig. 15). Some evidence of nestling behaviour is shown by the fact that clutches of eggs have been found in shallow depressions and eggs had been found carefully laid in concentric rings of 12–15 before being covered with sand. This is exactly what is done by modern sea turtles or crocodiles. The salt water crocodile may deposit 40–90 eggs in nest. Of course something goes wrong sometimes, *e.g.*, the heat of the sun is not good enough to hatch them. The *Protoceratops* nests also contain eggs which failed to hatch. Presence of larger *Protoceratops* skeletons suggest that the nestling grounds were protected by the adults.

Discoveries in southern France in 1969 mention of fossilized egg-shells in addition to a large sauropod, *Hypselosaurus priscus*, but a definite association of the two had not been realised. Discoveries during 1930 which found a complete fossil egg led to further explorations. Now over a hundred eggs, some of them in original form, many crushed, are on record. The largest ones are roughly twice the size of ostrich eggs. These have now been attributed to *Hypselosaurus* with much confidence. So far these are the largest dinosaur eggs known anywhere.

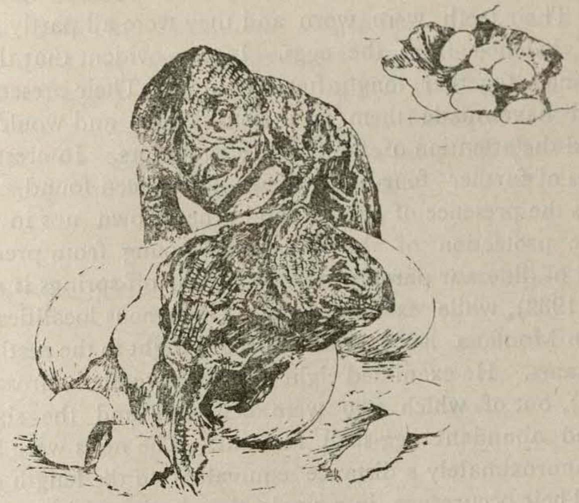


Fig. 15 The birth of a ceratopsian dinosaur (*Protoceratops*) from egg; nest is shown in the corner (from Jain, 1979).

An interesting study made by Seymour (1979) on Upper Cretaceous dinosaur eggs suggests that their egg-shells were highly porous. The nests were highly humid probably low in oxygen and high in carbon dioxide. Such conditions occured underground or within the incubation mound. In order to prevent depletion of oxygen and elevation of carbon dioxide to intolerable levels in the nest some sauropods were forced to limit their clutch size in each nest. The effect produced by modern mound-building birds (Thermometer birds) in the incubation mound seems similar to some dinosaurs.

Hadrosaurian Dinosaurs

The duck-billed dinosaurs are regarded as the most successful and well adapted among all the plant-eating dinosaurs, making up about 75% of the fauna. Many mummified specimens are known which give evidence of the texture of their skin. They inhabited a wide variety of environments: dark swampy cypress forest, open woodland of broadleaved trees or flood plains of large meandering rivers with low shrub-like vegetation. Some of the best known material has been found in Canada, U.S.A. China and Argentina.

Maiasaura peeblesorum, a hadrosaur, has been recognised in 1979 from the Upper Cretaceeous of Montana (USA). The entire material consists of specimens represented by partly grown juveniles about a metre in length. A nest mound, made from mud, about 3m (10 ft) in diameter was found. The nest was at the submit of a saucer-like depression excavated in a mound of about 1.5m (5 ft) high. The depth of the depression was about 75 cm (30 in). Within the nest were eleven immature hadrosaurs each about a metre long. Their teeth were worn and they were all partly grown so they had not just hatched from the eggs. It was evident that the young hadrosaurs remained together long after hatching. Their presence in a raised mound would have made them very conspicuous and would undoubtedly have attracted the attention of flesh-eating predators. Interestingly, outside the nest bones of further four individuals have been found. This is being interpreted as the presence of parents and other grown ups in the proximity of nest for the protection of the vulnerable young from predators. Such dramatic find of dinosaur parents protecting their off-springs is rare indeed.

Horner (1982), while examining late Cretaceous localities of hadrosaur nesting sites in Montana, has provided further insight to the nestling behaviour of these dinosaurs. He examined eight nests of juvenile hadrosaurs (undetermined species), out of which two were occupied and the six unoccupied nests contained abundant egg-shell remains. The nests were found at least 7m apart or approximately a distance equivalent to the length of an average hadrosaur. Their occurrence in a single time horizon within a small area is suggestive that these hadrosaurs were nesting in a colony. It has also been found that. after hatching, some species remained in their respective nests whereas others left the nest but retained it and returned occasionally to the same nesting site.

Juvenile Dinosaurs

Though many dinosaurs were giants they did not produce gigantic eggs. As stated earlier, the largest known eggs are attributed to a large sauropod, *Hypselosaurus*. The disparity in the size of eggs and the dinosaur which laid them is astonishing. However,there is bound to be an upper limit to the egg size based upon certain physical constraints. A newly hatched *Hypselosaurus*, about 40 cm long, has to grow about 30 times in length to reach an adult length of about 12 m. In comparison, the salt water crocodile begins as a miniature crocodile about 20 cm long and the adult is about 6 m long. The proportionate increase is exactly similar. In the case of *Protoceratops* the linear increase may be only 7–8 times.

Fossil remains of minute specimens of a ceratopsian dinosaur, *Psittacosaurus* ('parrot-reptile'), have been discovered in 1980 in Lower Cretaceous rocks in Mongolia and China. This dinosaur has sharp downturned upper jaw which gives the impression of the beak of a parrot. There is also the begining of a fill at the back of the skull. Each specimen in life would have a length of 25 cm (10 in) and a mass of about 0.7% of the adults. Although these are among the smallest dinosaurs known their teeth already show wear, which means that they were already feeding on fairly tough plant materials. Their minute size, compared with adults, indicates that parents could not have been directly concerned with caring for them, beyond being in the vicinity and protecting the nesting site from predators.

The smallest dinosaur ever discovered, however, is a *Prosauropod* from Upper Triassic beds in Patagonia, Argentina. Discovered in 1979, it appeared like a 'mouse among dinosaurs' and aptly named *Mussasaurus patagonicus*. The largest specimen was only 20 cm (8 in) in length. This dinosaur was very young individual and was found with some five others and remains of two eggs all associated in a nest. It showed that after hatching the young remained together in the nest.

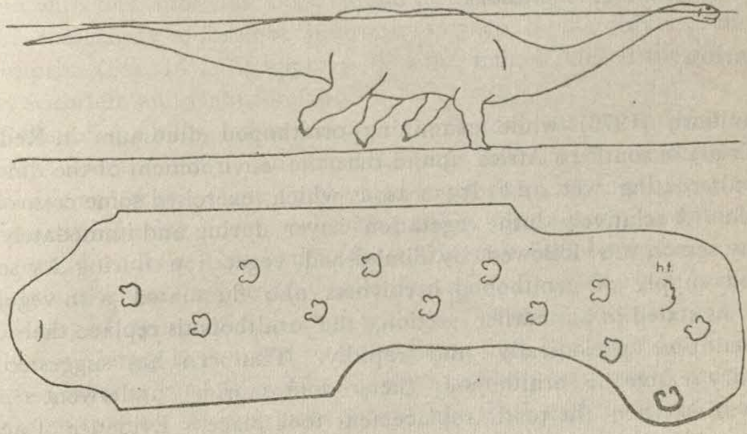


Fig. 16 Swimming habits of a sauropod dinosaur (above) are deduced from the trackway (below) consisting of forefoot prints with only a single hind foot (from Coombs, 1975, after Bird, 1977).

The known record of dinosaur eggs, embryos and juveniles is extremely scanty yet it holds much promise for palaeobiological studies. Except *Hypselosaurus*,definite eggs or egg-shell of better known sauropod dinosaurs have not been found. It would be of great biological interest to compute growth curve of such dinosaurs if there are physical limits to the maximum size of an egg. In India, as stated earlier, eggs and egg-shell material, including probable pathologic specimens (Mohabey, 1984a), so far known is from Cretaceous beds in Kheda district, Gujarat and Bara Simla Hill, Jabalpur (Sahni and Gupta 1982, Mohabey 1984). No juveniles have been found yet. Wherever a large deposit of dinosaur bones has been found anywhere the eggs are never found there. This may imply that, just like large modern reptiles, the nesting sites were usually away from the normal habitat of dinosaurs.

Habitat, Environment and Behaviour

Habitat, environment and behaviour of dinosaurs is known from a large number of studies and only mainfindinge can be examined within the scope of this article. The wide variety of habitats, the long geological range and morphological specializations of a number of genera make generalizations difficult. Evidence about aestivation, climbing and swimming capacity of some dinosaurs has recently become available. Possible sexual display, vocalization and advanced predatory behaviour of some dinosaurs and detailed environment of some dinosaur strata, such as Morrison Formation (Late Jurassic,) U.S.A., Oldman Formation (Cretaceous, Canada) and Tendaguru (Late Jurassic, Tanzania), has recently been recognised. Detailed studies on speeds and grits of dinosaur (Thulborn, 1982) have revealed astonishing conclusions.

Aestivation

Thulborn (1978) while examining ornithopod dinosaurs in Red Beds (Late Trias) in southern Africa found that the environment of the dinosaurs had an alternating wet and dry season which exercised some control over plant life. A relatively lush vegetation cover during and immedately after the rainy season was followed by diminished vegetation during dry season. The food supply of ornithopod herbivores also fluctuated with vegetation cover. As stated in an earlier section, the ornithopods replace their entire cheek-dentition periodically and rapidly. Thulbcrn has suggested that during dry season the ornithopods (heterodontosaurids) underwent a period of aestivation when the tooth replacement took place. Evidence of aestivation is cited in the nature tooth replacement and absence of worn and discarded teeth in Red Beds. Some Red Bed dinosaurs (*Fabrosaurus*) found in association had virtually sharp and unworn teeth in the jaws. These were found in accumulation of worn and shed teeth. It has been suggested that this assemblage comprises of animals that perished during aestivation. It may be mentioned that crocodilians also aestivate or hibernate.

Climbing Ability

Hypsilophodon, a particularly primitive ornithischian dinosaur, is known by several excellently preserved skeletons from the Lower Cretaceous of England. It is a bipedal herbivore. Galton (1971, 1971a) found that in this dinosaur the metatarsal—I was closely applied along its length to metatarsal—II, and the first digit moved parallel to or slightly away from the other digits. Relative to the trunk, the hind limb is long, with an elongate tibia and metatarsus. These proportions fall in the range for cursorial mammals. In addttion, a rigid tail was an aid to balancing. Dermal armour being light only provided inadequate protection frcm predators. It si not suggested that cursorial mode of life was normal but individuals did occasionally go up onto the trees mainly to keep away from predators.

Swimming Ability

Direct evidence for the swimming ability of dinosaurs is necessrily rare but wherever available it is convincing. Although sauropod dinosaur feet were ill adapted to swampy or muddy terrain, yet evidence in the form of footprints indicates that sauropods, like elephants, could and did cross muddy and swamp ground. The better known sauropod trackways near Glen Rose, Texas, appear to be made in mud sufficiently soft to allow huge sauropod feet to sink to depth of 30–35 cm (Coombs 1975). Evidence about some sauropod dinosaurs floating the hindquarters while "walking" along the bottom with forelimbs has been found in the trackways which are composed almost exclusively of forefoot impressions, there being only one single hind foot imprint (Fig. 16). It appears that the animal kicked the ground alternately with left and right forefoot.

Swimming ability of carnivorous dinosaurs has been evidenced only recently (Coombs 1980). In general, large lakes and streams have been visualized as escape route for harried vegetarians, especially sauropods and hadrosaurs, inferring lesser swimming ability for pursuing predators. However, unsual tracks at Connecticut State Dinosaur Park have been uncovered recently which were apparently made by a partially submerged theropod that was kicking along a muddy bottom with the tips of its toes. Tracks indicate a tridactyl large biped and were made on firm or almost dry mud or on a very thin layer of mud (Fig. 17). Only a partially submerged animal could make such tracks. *Eubrontes* was possibly the track maker of such footprints.

Sexual Display

The horned dinosaurs or ceratopsians had their origins in northeastern Asia but much of their evolutionary development is recorded in western North America. Among the better known forms is *Triceratops*, reaching upto 9 m (30 ft) in length and one of the most successful dinosaurs at the very end of the Cretaceous. It comes from Lance Formation (Upper Cretaceous) in U.S.A. and Canada. The other is *Pentaceratops* having a huge frill and five horns, also about the size of *Triceratops*, and has been found in Upper Cretaceous (Fruitland and Kirtland beds, U.S.A.) rocks. The function of horns has been a subject of much discussion in these ceratopsian dinosaurs. In all probability, these were potent weapons against attack from giant predatory dinosaurs. The long frill was likely used as a frontal threat display. However, it has also been suggested that the horns were mainly used in contests between members of the same species. Sexual combats between individuals for the supremacy would have the rival males lock their horns and push and shovel one another until one of them conceded defeat.

Such a situation is commonly seen in mammalian antlers and it is quite concieveable that it might have occurred in ceratopsian dinosaurs as well.

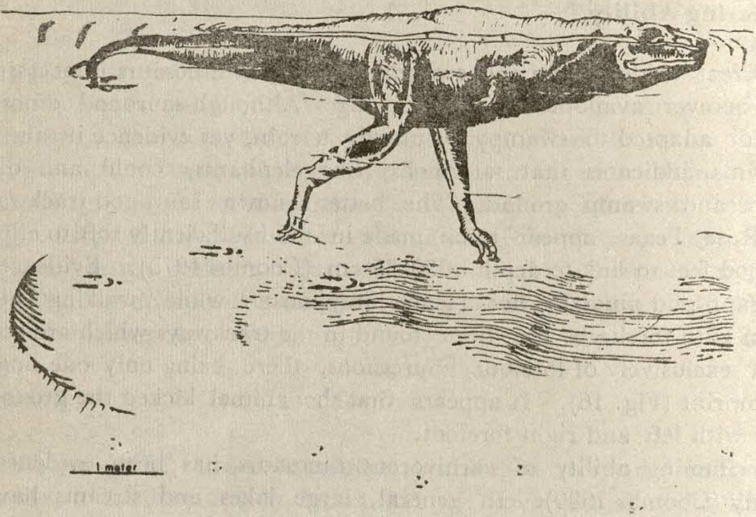


Fig. 17 Swimming habits of a carnivorous dinosaur (above) are deduced from the trackway (below) in Lower Jurassic rock and Rocky Hill, Connecticut, USA (from Coombs 1980).

Vocalization

Among the Late Cretaceous hadrosaurs two groups are recognisable based on the shape of their skulls. The Hadrosaurinae have either solid crest or lack them altogether and Lambeosaurinae bear hollow crests containing portions of nasal cavity. In a recent study, Weishampel (1981) has attributed resonation properties to the Lambeosaurine hollow crests as a result of acoustic analysis. Size and shape of nasal cavity suggests low vocal frequencies in adults which is compatible with potential auditory acuity in these animals. The disposition of lateral diverticula in juveniles and adults indicates that the former vocalized at higher frequencies than the latter. The potential for high frequency auditory sensitivity in adults suggests a high degree of parent-offspring vocal communication. In some lambeosaurine dinosaurs there appears sexual dimorphism in the crest size of male and female which indicates different resonant properties of the nasal cavity and different vocal ranges. Low frequency vocalizations, it appears, allowed lambeosaurines to exploit intraspecific communication in both open and closed habitats. These have further advantage of attenuating less rapidly over long distances than do high frequency signals. Close parent offspring ties, as in crocodiles, were possible in these dinosaurs largely because of vocalization ability. It may be noted that crocodiles are known to be particulary noisy, especially during mating season, when they bellow and roar in what is sometimes described as a growling rumble.

Predatory Behaviour

Among the carnivorous dinosaurs, an important group developed remarkable specializations during Cretaceous which make them distinctive from all others. These were all lightly built deinonychosaurs which developed an enormous claw on the second toe of the hind limb which acted as a weapon for tearing open their prey. The long clawed hands were specialized for grasping and holding. When considered in conjunction with the huge sickle-shaped second toe which was an organ devoted solely to slashing and tearing, a picture emerges of an active killer dinosaur, entirely distinct from ponderous giant scavangers. In an earlier section, an account of Lower Cretaceous *Deinonychus* (Fig. 7) has been given. The agility of this dinosaur was completely unexpected and the tempo of life was vastly more energetic than previously imagined.

Velociraptor, an Upper Cretaceous Mongolian deinonychosaur, is rather a small dinosaur. It also retains the important sickle claw as its major weapon for dealing with its prey. A complete skeleton of this dinosaur was discovered in 1971 in Mongolia in association with the herbivorous *Protoceratops*. Both had perished at the very moment of *Velociraptor*'s attack on *Protoceratops*. The analysis of two skeletons suggests that *Velociraptor* was grasping the head of *Protoceratops* in its hands; the sickle claw of the foot was in the very act of tearing into the body.

Saurornithoides from Mongolia and *Stenonychosaurus* (Fig. 18) from Canada are the last representatives of deinonychosaurid dinosaurs. Their arms had more manoeuverability than those of other related dinosaurs. The skull was much longer with small serrated teeth, but the most obvious features were the enormous eyes. The size and position of the eyes were such that both the eyes were able to focus on the same object, *i.e.*, binocular vision. The animals preyed upon lizards and small mammals. The size of eyes suggests that they hunted during twilight hours when light was fading. As well as the enormous development of the eyes was the notable development of brain. In contrast, the casts obtained from known cranial cavity of other dinosaurs indicate small brains (Fig. 19), and the one recently described from India (Berman and Jain 1982) is so small that it fits into human palm comfortably. The brain-to-body ratio of these deinonychosaurid dinosaurs falls completely outside the range of all living reptiles and dinosaurs and is closer to early mammals and present day flightless birds. As compared to crocodiles, the deinonychosaurs have nearly 6 times brain power. These dinosaurs have achieved much fame as being the most specialized and advanced. They have also inspired the characterization of a highly successful movie, E.T.

Environment

Early dinosaurs were active bipedal predators which appeared in Late

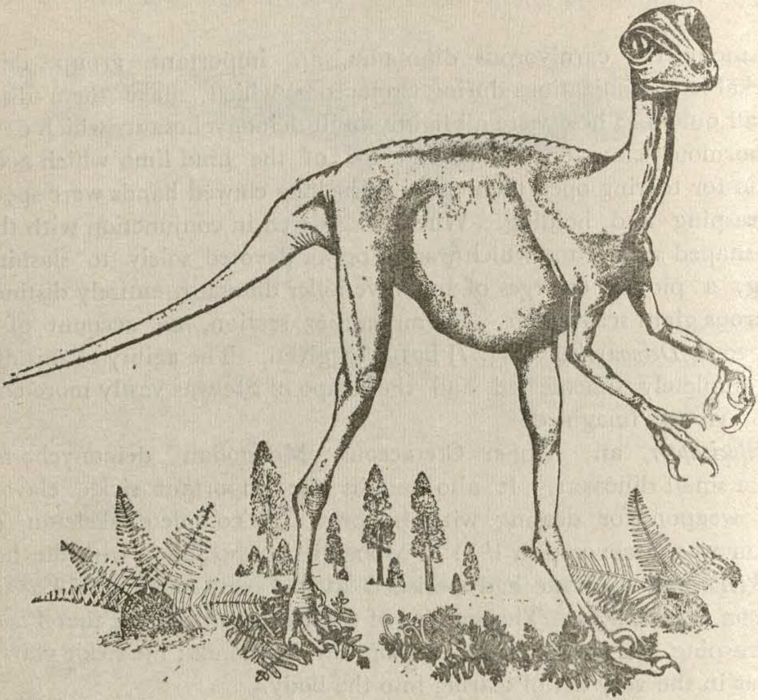


Fig. 18 A restoration of Late Cretaceous (Lance Formation) *deinonychosaurid* dinosaur, *Stenonychosaurus*, in the background of Lance vegetation (from Russell, 1982).

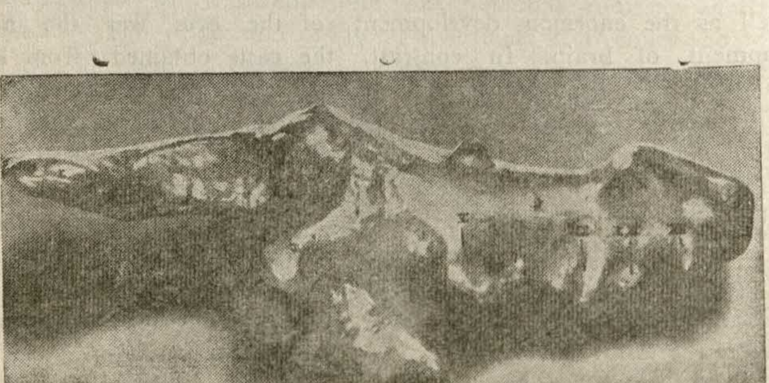


Fig. 19 A cast of brain cavity of *Triceratops*. an Upper Cretaceous horned dinosaur of Wyoming. Actual size : 15.5 cms from charig. (1979).

Triassic times. Some of them hunted in packs as indicated by analysis of footprints. This period is notable for the extensive desert areas in the world and marked seasonal droughts. Some dinosaurs even hibernated as indicated earlier. However, there were areas with lush jungles in which there were numerous streams, swamps and lakes as well. During Jurassic times, a great sea covered western North America and parts of Europe. Small restricted areas where dinosaur fossils have been found indicate partly desert, partly swampy conditions, with tropical foliage providing protection for the giant dinosaurs. The Lower Jurassic sauropod dinosaur from Kota Formation, India, *Barapasaurus tagorei*, was inhabiting land close to Kota lake and largely fed upon nearby vegetation (Jain 1980). In Morrison Formation, the dinosaur environment is suggestive of well-drained floodplain, wet overbank and shallow backswamp conditions (Dodson, Bahrensmeyer & Bakker 1980). During early Cretaceous, there was possibly slight drop in world temperatures but it did not last long. For most of the Cretaceous, warm climates followed widely as in Jurassic time.

The Late Cretaceous floral and faunal assemblage has received much attention because of the decline and extinction of dinosaurs. In Montana (USA) moist subtropical woodland is gradually displaced by open woodland dominated by pines toward the end of Cretaceous (Valen and Sloans 1977). In Alberta (Canada), evidence of prevailing subtopical climates with well watered basin having high rainfall has been found. A few angiosperms, conifer fronds and cone, taxodiaceous trees and metasequoian foliage prevailed (Beland and Russell 1978). In Tendaguru (Tanzania) sediments indicate that water was abundant at least locally; palynofloral and sedimentary evidence indicates alternating dry and wet season (Russell, Beland and McIntosh 1980). Ostrom (1964) made an analysis of Late Cretaceous environment related to hadrosaurian dinosaurs in U.S.A. and found that the landscape was dominated by rich and diverse lowland forests of conifers, poplars, willows and oaks. He found little evidence of aquatic plants and suggested that hadrosaurus were actually active terrestrial foragers.

Speed and Gait

Speculations about locomotor abilities of dinosaurs are numerous but the most noteworthy estimates are recent ones (Coombs 1978, Thulborn 1982). Most speculations are based on comparative anatomy and on theoretical predictions based on the principles of biomechanics. In many instances, relative locomotor abilities of dinosaurs rather than absolute speeds are available and there is wide discrepancy in various estimates. As such, justifiable caution must be exercised in accepting the figures of absolute speed. Attempts at estimation of speeds are related to gait, height and weight of various dinosaurs. In Table II, height and weight of selected bipedal and quadrupedal dinosaurs has been given. It may be noted that the Table includes wide range of dinosaurs, weighing as small as 3 kg to much as 78,000 kg.

From the study of locomotion in living terrestrial vertebrates, Alexander (1976) obtained a relationship between speed, body size and stride length. This relationship has been applied to dinosaur trackways where stride length could be measured directly and the size of the dinosaur cold be estimated from the size of its footprints (Mossman and Sarjeant 1983). Table III gives the top running speeds (stride length and speed) of selected dinosaurs. It may be seen that both in stride length and speed the bipedal dinosaurs can overrun the quadrupedal dinosaurs, the small carnivores (*Deinonychus* and *Dryosaurus*) being the fastest. It must, however, be remembered that, just like modern carnivores, this speed could be sustained for a short while e.g., to pursue a prey. Table IV gives estimated speeds of related dinosaur groups during walking, trotting and running. It may seen that in many cases a running speed of almost 10 times of walking speed can be achieved. The swiftest dinosaurs were most probably the small and gracile members of Ornithopoda and Theropoda. Bigger bipedal dinosaurs also achieved considerable speeds. The great majority of quadrupedal dinosaurs (sauropods, stegosaurs, ankylosaurs and ceratopsians) seem to have been restricted to walking gaits (Fig. 20)

Theoretical speed estimares obtained suggest that some dinosaurs could have approached, or even bettered, the locomotor performance of mammals. However, the estimates given above indicate that mammals would usually have outstripped dinosaurs (on weight basis) though the general relationship between speed and weight is similar in the two groups. If a dinosaur could match tne running performance of a mammal, one would expect it to have attained mammalian level of efficiency in locomotor anatomy and in its physiology. Though direct evidence is lacking there is sufficient anatomical data to suggest that *Dinonychus* and *Stenonychosaurus*, with the highest brain-to-body ratio among reptiles, could have achieved mammalian speeds.

The Great Extinction

The Background

The dinosaurs along with various other reptiles became extinct at the end of Cretaceous, some 65 milion years ago (Fig. 21). Not one of them survived into a later geologic age as no trace of a dinosaur bone or tooth has ever been found in any post-Cretaceous rock, not even in the earliest of them. The geological record on this score is irrefutable.

The disappearance of the dinosaurs along with other reptiles with which they were contemporaneous has engaged the minds of scientists all over the world. Yet the problem remains unsolved, although new aspects to it are being added from time to time. Some suggestions which appear attractive initially do not stand the controversial evidence otherwise

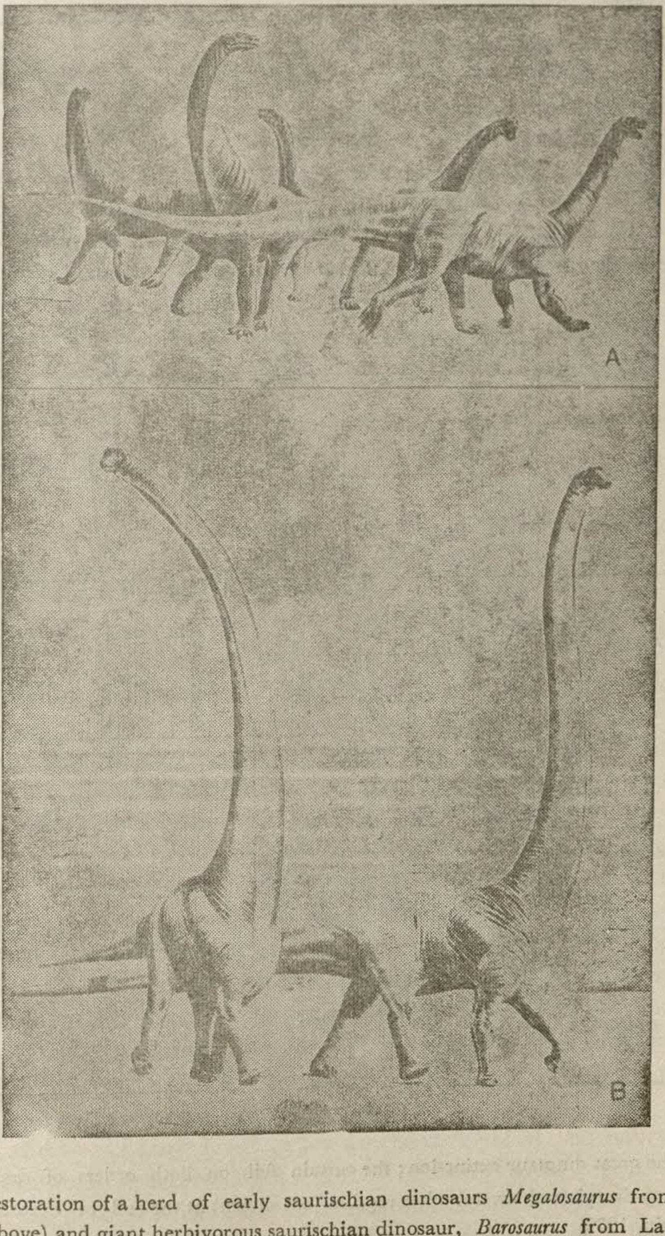


Fig. 20 Restoration of a herd of early saurischian dinosaurs *Megalosaurus* from Lesotho (above) and giant herbivorous saurischian dinosaur, *Barosaurus* from Late Jurassic of North America and Africa (from Crompton, 1968; restoration by R. T. Bakker).

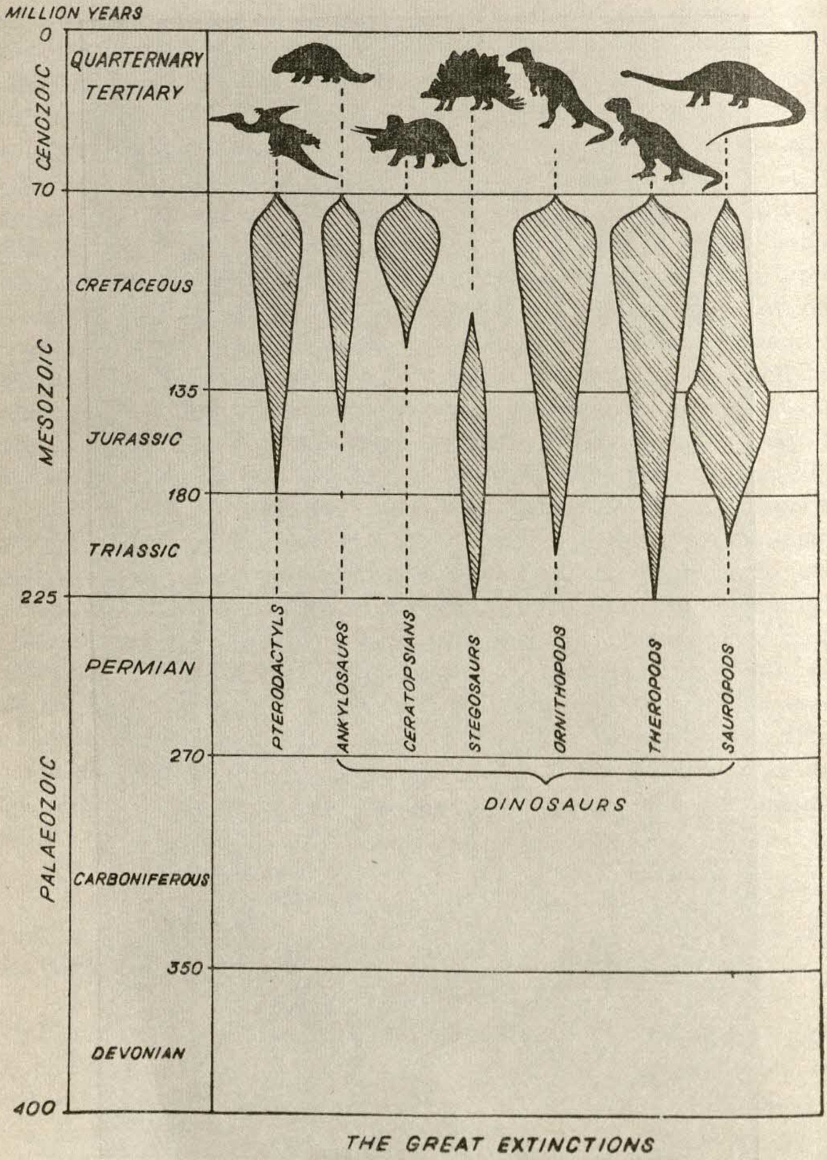


Fig. 21 The great dinosaur extinction; the curtain fell on both orders of dinosaurs and pterodactyls and several other reptiles (not shown) at the end of Cretaceous (from Jain, 1979).

available. Basically extinction can be defined as the inability of life to adapt itself to changing conditions. It seems logical, therefore, to examine the great change that took place at the end of Cretaceous times, which brought to an end the multitudes of dinosaurs and other reptiles that populated the earth.

One of the best places in the world for the study and excavation of Upper Cretaceous dinosaurs is western North America where a sequence of successive deposits containing vast quantities of dinosur bones have been found. The succession of deposits represents the last few million years of Cretaceous history with the top (Lance Formation) showing the very close of the Mesozoic events. There is a sharp decline in the abundance of dinosaur species somewhat before the end of Cretaceous time (Pre-Lance period) to the extent the sauropods had already become extinct in the Lance age and theropods, ornithopods, ankylosaurs and ceratopsians had become much reduced in numbers. It can, therefore, be said that the process of extinction extended over a considerable period of time, say 5 million years, before the curtain finally fell on the dinosaur scene.

Causes of Extinction

At one time, not long ago, a world-wide catastrophe was regarded as the reason which wiped out the dinosaurs. Geologists have never found any evidence of such a disaster. On the other hand, the history of the earth and its life has advanced through the ages by a grand succession of events. There have been a series of earth movements that marked the birth of modern mountain system – the Himalayas, the Andes, the Alps and the Rockies during the latter portion of the Cretaceous history — usually referred to as Laramide Revolution. The massive mountain uplifts affected climates and plants which may have had an adverse effect on animals including dinosaurs. There is a good deal of evidence for profound changes in plant life that took place during Cretaceous times which may be, in part, a result of Laramide uplift. Dinosaurs that lived at the advent of Cretaceous history inhabited a world of primitive plants ; dinosaurs of the late Cretaceous inhabited 'modern' forests composed of familiar broad-leafed trees, conifers and palms with the intermingling of primitive plants. The important point here is that hordes of dinosaurs did live in this changed world for many millions of years. The post-Cretaceous world does not seem very different from the late Cretaceous world. Then why did not at least some of the dinosaurs survive ?

The effect of changing vegetation on the survival of dinosaurs has been recently examined in a new light. Dr. T. Swain of the the Kew Royal Botanical Gardens in Britain has suggested that nature killed dinosaurs by introducing into their diet a new class of aromatic type of alkaloids. The dinosaur were tricked by the chemistry of the new plants and died of alkaloid food poisoning. In the evolution of plant kingdom of earth, first came the flora free of alkaloids. They were harmless to dinosaurs which flourished and dominated the earth. Then came Angiosperm with their ability to synthesise aromatic type of alkaloids. The dinosaurs unable to detect such compounds probably ate enough to suffer severe physiological disturbances and even death. Swain conducted some experiments on the Mediterranean tortoise-one of the few remaining vegetarian reptiles. He found that the tortoises continued to eat the stuff even at alkaloid concentration 250 times higher than those that would stop mammals from eating it. The dinosaurs might have done the same thing, and died. They were unable to sense the poison in their diet and were not capable of defending themselves against the "chemical aggression". It is further asserted that disappearance of the 12 families of vegetarian dinosaurs was followed by a rapid decline of the remaining families of carnivorous dinosaurs because the principal diet of these reptiles was their vegetarian cousins.

Warm-blooded Dinosaurs

Dinosaurs had so far been regarded as peabrained, cold blooded creatures that spent most of their lives hulking sluggishly in the sun. This concept has drastically changed in last few years with the ideas propounded by R. T. Bakker (1975) of Peabody Museum of Natural History, Adrian Desmond (1975 of Harvard University and L. Van Valen and R. E. Sloan (1977) of Chicago and Minnesota University, respectively. It is now believed that at least some dinosaurs were warm-blooded, complex and far more intelligent than some of the species that succeeded them. The extinction of dinosaurs in the back-ground of the fact that there was worldwide lowering of average temperatures during Laramide Revolution may now be examined. It is argued that the cold-blooded reptiles such as turtles and crocodiles were able to find cozy nooks and corners and survive the winter by hibernation: small fur-covered mammals built warm nests which the dinosaurs could not. Too big to find protective caves or burrows in which to hide, they stayed out, and died.

One Russian school of thought suggested sometime back, that increased amount of radiation might have caused the extinction of dinosaurs. Investigation of volcanoes which are active at present goes to show that during the period of volcanic eruption a great amount of radioactivity is observed. Present volcanic activity is quite insignificant as compared to the gigantic volcanic activity in the past. This is testified by the basaltic discharge of the Tulle, Siberian and Deccan Traps, the gigantic lava plateau of Columbia and patagonia, the South African dolerite of Karroo, etc. The harmful effects of radiation are known to accumulate. Even very small doses of ioinsing radiations exert harmful effect on an organism giving rise to mutations which are often lethal for the organism and render it sterile. The cumulative affect of such exposure may have caused extinction. The basic question still remains: If the effect of radiations was so lethal, how could other forms of life out-survive into the post-Cretaceous times?

Competition with Mammals

Competiton of dinosaurs with mammals has been suggested by some scientists as the cause of the demise of the former. It appears an unsatisfactory explanation because mammals were relatively rare and small in the late Cretaceous times, and most of the Palaeocene ones were smaller than the majority of the dinosaurs, so that their ecological requirements were probably different. Mammals did not begin to spread and produce species until after the dinosaurs had become extinct at the end of Cretaceous. One is, therefore, forced to conclude that dinosaurs were competitively superior to mammals as large land vertebrates.

If the mammals did not actually compete with dinosaurs, is it not possible that they brought about the end of the great reptiles by robbing dinosaur nests and eating dinosaur eggs? This has been seriously considered as an important factor and could have decimated dinosaur populations, but it seems unlikely that such forays against hatching eggs could have wiped out dinosaurs. The nests and eggs of various modern reptiles, such as turtles, are subjected to intensive onslaughts by animals, not the least important of which is man, but the reptiles continue. And if any of them does disappear it will be due to man's greed and not through 'natural' enemies.

Were the dinosaurs exterminated by great epidemics that swept the Cretaceous world? This is hard to believe. Experience with man-made or propagated diseases suggests that many animal species have varied resistance to disease and that, when epidemics occur, the disease-resistant members of the population survive. Moreover, the effect of the disease depends on the intensity, speed and degree of coverage which strikes a population, the number of disease-resistant members and their reproductive capacity. To attribute the exticition on a world wide basis due to epidemics would be to stretch the credulity far beyond the bounds of reason.

There is yet another theory that 'racial senescence' hed set in the dinosaurs resulting in non-viability of eggs; this caused their extinction. In support, the discovery of a large number of eggs and egg fragments the Upper Cretaceous deposits of Southern France is quoted. A large number of eggs in these deposits are unhatched. It is argued that something was interfering in reproduction in the last of the dinosaurs. If the eggs had been so sterile as to bring about the extinction of the dinosaurs in this region, one would expect them to be of limited occurrence. This could hardly explain the worldwide extinction.

The situation is complicated by the fact that the two orders of dinosaurs, Saurischia and Ornithischia, were not the only reptilian orders which became extinct by the end of the Cretaceous period. The pterodactyls also disappeared, and so did two orders of marine reptiles, the dolphin-like ichthyosaurs, and the plesiosaurs. The marine reptiles were worldwide in their distribution, and were probably dwellers in open waters and were excellent swimmers. Migration in face of poor local conditions could have presented little difficulty to them. All the five orders of reptiles did not become extinct at the same time. All were present in the latter half of the Cretaceous period, but the ichthyosaurs seem to have disappeared first, then the plesiosaurs and pterodactyls, and finally the two dinosaur orders together with some enormous marine lizards belonging to the family of mosasaurs. The Cretaceous period was long geologically, and there might have been an interval of anything upto 20 million years between extinction of ichthyosaurs and the end of dinosaurs.

The Extra Terrestrial Causes

The most recent and widely publicised theory about extinction has attributed it to extra-terrestrial event (Alvarez *et el.* 1980). This includes the explosion of a supernova in nearby space and the collision of a huge meteor or an asteroid with the earth -with complex results (Russell 1982).

The centre of the argument revolves around presence of Iridium in a layer of clay in certain late Cretaceous marine limestones, one to two centimetres thick, overlain by marine limestone of early Palaeocene age. Iridium is concentrated in the layer of clay only in which no organisms are preserved. In the limestones below the clay layer are typical marine organisms of the latest part of the Cretaceous. In the limestone above the clay the late Cretaceous organisms are absent; they have been replaced by other organisms typical of the Palaeocene.

Iridium is rarely present in the rocks of the earth's crust but is comparatively abundant in meteorites. The steady rain of micrometeorites on the surface of the earth results in modest concentrations of Iridium which accumulate in the ocean basins. The Iridium concentration found in some clay sample of late Cretaceous age was about 30 times more abundant than clays from adjacent limestone strata. Confirmations have been obtained from Italy, Denmark, Spain and New Zealand. It is suggested that the impact of an asteroid, explosion of supernova, sudden changes in oceanic circulation and composition or atmospheric perturbations have resulted in biotic catastrophe.

The impact of an asteroid, some 6 to 14 km in diameter, produced a dust cloud that obscured the sun and stopped the photosynthesis for several years and there by caused the extermination of many groups of organisms. As it settled, this dust, distinguishable by its relatively high concentration of iridium and other platinum group elements, was incorporated into both marine and terrestrial sediments.

Critics of this hypothesis (*e.g.*, Clemens 1981) have argued against invoking some kind of catastrophic, short, sharp shock as the causal factor of the terminal Cretaceous extinctions. The palaeobiological data suggest the

Cretaceous - Tertiary transition was a period of several tens of thousands if not hundreds of thousands of years of duration, characterissed by interaction of complex physiological and biological factors producing a high net rate of decrease in biotic diversity within both terrestrial and marine biotas. As such, it appears that the curtain fell slowly rather than suddenly to close the Cretaceous chapter.

Concluding Remarks

Our understanding about dinosaurs has come a long way since their scientific recognition about 150 years ago. The excitement and enthusiasm in finding bones of giant reptiles in different parts of the world in the early history has yielded ground to learning about them as living animals. What were once considered 'giants' and 'terrible' are now known to be not necessarily so. With the discovery of small dinosaurs, the myth of their giant size has been broken. *Tyrannosaurus* so far considered 'King of terrible lizards' has now been assessed to be a mere scavanging reptile.

Giant strides have been made in the knowledge of dinosaur anatomy and physiology largely because of multidisciplinary approach. Comparisons with living reptiles and mammals, studies on palaeohistology, predator-prey relation of modern environments and its comparison with fossil population have yielded a new insight into dinosaur life. The debate on thermal regulation has opened new vistas of research which has been recently summarized (Jain 1981). Dinosaurs which were once considered singularly unsuccessful reptiles are now considered highly successful (Colbert 1983). They would not have dominated the earth for 135 million years, it is argued, if they were not successful. Their geological history is far longer than many other reptilian groups.

Extinction of dinosaurs still continues to be in the forefront of debates. Ever since the finding of dinosaurs, there never was a dearth of theories and explanations. However, each theory had a big snag which prevented it from receiving general acceptance. With the arrival of evidence of possible endothermy in dinosaurs a new twist was given to extinction theories (Jain 1978) but the conclusions are still far from satisfactory. The decade of eighties has ushered in extra-terrestrial element in extinction theories. The subject has been debated in recent Workshops and Seminars, and although there is more solid evidence, the contrary evidence still baffles scientists.

The basic trend during the current and next decade, in addition to finding new dinosaur sites and bridging the gaps in dinosaur evolution, is expected to lay emphasis on anatomy, physiology, musculature, food and feeding habits, growth and development and eco-environment of dinosaurs. It is already quite apparent that dinosaurs were different from other living reptiles and this distinction is likely to become more profound. Bakker (1975), who proposed a new class of vertebrates for the dinosaurs, rather prematurely, may be after all quite justified in his suggestions. The new lines of research in dinosaur studies are almost endless. Each new theory opens up new lines of investigation which calls for collaboration of allied sciences. The current knowledge is not only helping to understand the dinosaurs better but is generally improving our understanding of other reptiles, eary mammals and their relation to environment.

Acknowledgements

It is a pleasure to acknowledge the untiring efforts of Dr. Pamela L. Robinson (University of London) in leading exploration and excavation teams during 1959–62, Indian Statistical Institute, Calcutta, which enabled India to join the few selected countries having mounted dinosaur skeletons. Dr. Edwin H. Colbert's (American Musem of Natural History and Museum of Northern Arizona, Flagstaff) association with our dinosaur research, his participation in excavation work in 1964 and critical comments during actual mounting in 1977 have been a source of much encouragement. Palaeontologists on shorter visit include Prof. T. Stanley Westoll, F.R.S. (1964), Dr. E.I. White, F.R.S. (1964), (late) Prof. Alfred S. Romer (1971) and Dr. J.W. Cosgriff (1982). All have been a source of inspiration.

A number of illustrations appear in the article from sources indicated in the legend. Grateful acknowledgements are extended to the publishers for permission to reproduce them.

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4

AGING

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Introduction

The life span of multicellular organisms may be broadly divided into three phases : growth, reproduction and senescence (aging). During growth, an increase in the number and size of cells and their differentiation to perform specialised functions occur. The sizes of organs and organisms increase and reproductive ability is attained. The reproductive phase is marked by the appearance of factors necessary for reproduction such as hormones. Organisms which do not reach reproductive phase are of no consequence for the perpetuation or evolution of the species. The rate of reproduction is high initially and then decreases gradually. Faster the reproduction rate, shorter is the generation time of a species. Or, higher the number of offsprings produced, shorter is its maximum life span. For exampie, mice and rats reproduce much faster than larger mammals like elephants and horses and also have shorter life span. It appears as if reproduction depletes the organisms of some essential substances which are not replenished as fast as they are lost. Whether this loss is the cause of senescence and shortening of the life span is not known. The duration of this phase is more or less defined, particularly in females. In rats reproductive ability is attained at 10 weeks and ends at about 60 weeks. In the human female it starts at about 12 years and ends at about 45 years.

Aging is a characteristic of all multicellular organisms. It is marked by a decline in functional ability of the organism which becomes perceptible even towards the latter part of the reproductive phase. Thus the reproductive phase smoothly merges into the senescence phase. Reproduction does not occur during senescence and the activities of all organs decline. Several changes occur at molecular levels resulting in decreases in the functions of organs and of the whole organism. The duration of senescence is not well defined because it is not known at what stage of the life span deterioration of functions begins. If cessation of reproductive ability is used as a criterion, then it begins at 60 weeks in female rats, and at age 45 in human females. But it is common knowledge that in human males and females, several functions like muscular activity and respiration begin to decline even from age 30. The senescence phase is of little consequence for the perpetuation and evolution of the species.

Thus, growth, reproduction and senescence occur in a sequence. The time of onset, duration and rate of senescence is dependent on the reproductive phase and those of reproduction are dependent on growth. The three phases are interrelated. Hence senescence or aging should not be considered as an isolated or independent phase of the life span. Information on development and reproduction may help in understanding the mechanism of senescence (Kanungo, 1980).

Human Aging

Longitudinal studies carried out by N.W. Shock at National Institute of Aging, Baltimore, U.S.A. on various physiological functions of 500 male volunteers at increasing age beginning from age 30 up to age 90 have shown that different functions decline at different rates. The activity of each function at age 30 was taken as the peak value, though it is possible that some of the activities may have their peaks at earlier ages. The functions measured were physiological and hence reflect gross changes in the activities of cells and organs. It is likely that molecular changes leading to these physiological changes and decline in overall performance in humans occur even earlier.

Some of the functions measured were: conduction velocity of nerves, basal metabolic rate, cardiac index, glomerular filtration rate, vital capacity of lungs, renal plasm flow and maximal breathing capacity. All these functions decrease with age. The rate of decrease of conduction velocity is the slowest and that of breathing capacity the highest. This shows that even though the maximum life span of individuals of a species is more or less fixed within a certain limit, functions of different organs begin to decline at different ages of the organism and the rates of decline are different for different organs. Furthermore, the rate of decline increases with increasing age, that is, the rate of decline of overall function between age 60 and 70 is greater than between 50 and 60, and the rate of decline between age 70 and 80 is greater than between 60 and 70. So the decline in overall activity gets accelerated with increasing age due to cumulative effects of the decline in function of different organs or cells in the earlier age. So there is a continuous acceleration of functions once they get started. Hence the overall rate of decline of the organism appears exponential. It is not known when exactly the decline in function in each organ begins. The decline is not uniform for any organ function, and shows considerable variability among individuals of the same species, both in rate and magnitude.

Furthermore, the adaptability to external and internal stresses decreases during senescence, the homeostatic mechanisms deteriorate and susceptibility to diseases increases. Death occurs at some point during this phase not because all the functions reach zero level of activity, but because of one or more diseases or afflictions which affect one or more organs so seriously during this period that recovery is not possible.

No function or parameter has yet been identified that begins to change from a fixed age and at a fixed rate in all individuals of a species in a given environmental condition. So it is not possible to define aging in terms of a specific parameter. The only parameter used by Biologists is the chronological age as an index of aging, but this criterion can be very erroneous since there are men of 60 who look like men of 40 and *vice versa*. Because of the lack of any definite knowledge of the cause of aging, rather descriptive definitions of aging are used such as "aging is a gradual decline in the adaptability of an organism to its normal environment following the onset of reproductive maturity" (Comfort, 1964), or "aging is a process that makes the organism more susceptible to diseases following the attainment of reproductive maturity" (Strehler, 1977).

Cell Death

Several events like gastrulation, birth and reproductive maturity that occur during development are well timed. These are necessary for production of a reproductively viable organism, and are necessary for perpetuation of the species. Hence they have been selected during natural selection. During these developmental stages, there also occurs cell death, *e.g.* localised cell death in the limb bud of a chick embryo, proximal end of the wing of insects, tail of the tadpole of a frog, pronephros in higher vertebrates.

This is necessary for formation of specific functions and a viable organism. Such cell death that occurs during development, particularly before the attainment of maturity should not be taken to mean that the organism is aging while undergoing development, because the death of these cells leads to the formation of a functional organ and an active, vigorous and reproductively viable organism, and not an old animal. Hence the view expressed sometimes that aging occurs even during development is not justified because any death of cells necessary for the formation and effective functioning of an organ cannot at the same time cause aging of the organ or the organism. Death of cells that occurs after organs have been fully formed and the organism has attained maturity is taken as a sign of aging of that organ, as for example, death of neurons, and cardiac and skeletal muscle cells.

Significance of the Problem of Aging

In the early part of this century, infectious diseases like respiratory and intestinal diseases, accounted for most of the deaths in humans. With the advent of antibiotics and other advances in Medical Sciences, these diseases have been more or less controlled. This has caused a great increase in the average or mean human life span from age 30 to age 52 in India, and from 40 to 72 in developed countries. The control of these diseases has not, however, made man escape aging and death. The main killers of man during the last two decades are cardiovascular diseases, cancer and cerebrovascular diseases, in the order mentioned. These impairments appear to be intrinsic and are called "old age diseases".

The rapid strides made in medical sciences have given hope to man to reach his maximum life span of about 100 years. 12-15% of the population in developed countries is over age 60, while the average life span is about 72. By the year 2000, it will be about 20%. In the developing countries including India, lack of proper health care, failure to control infectious diseases, malnutrition and undernutrition have kept the average life span at about 50, and only about 6% of the population is over 60. This percentage is continuing to increase as medicare is improving. One of the effects of birth control and attempts to attain zero growth rate, particularly in developed nations, is that the population is getting old and a smaller and smaller fraction of younger people shall be supporting an increasingly larger proportion of old people in the future. Older people are susceptible to diseases, are physically less capable of carrying out their work and need care-financial, health and social. The rapid increase in the number of old people in every nation and the problem associated with it have generated a sense of urgency and interest in all nations to tackle various aspects of the problem of old age. The United Nations organised a "World Assembly on Aging" from July 26–Aug 6, 1982 in Vienna to take stock of the problems arising out of the rapid increase in the population of old people in all countries. 124 countries including India participated in the conference. The conference made recommendations for intensive research on the Biological, Medical and Social problems of the elderly.

The Biologists interested in the problem of aging are studying the molecular, biochemical, biophysical, physiological and structural changes occurring in various animals after they attain reproductive maturity with the aim of understanding and identifying the cause of aging. This may help in developing methods to postpone or defer the onset of old age, if not prevent aging altogether. The aim of the researchers is "to add life into years, not years into life".

Aging is a universal phenomenon, and the discovery of the cause of aging will be a fundamental finding. The problem is intellectually challenging. The important questions that have confronted these researchers are: Why do all organisms undergo deterioration of function after attaining reproductive maturity ? Why do all members of a species have a more or less fixed life span ? Why does a rat live for three years, an elephant for 70 years and a man for 100 years ? At what age after attaining maturity does the process of aging begin ? Is there a trigger, a switch, which sets in motion the process of deterioration ? If so, how is it switched on ? Is this proces programmed just as the process of development is ?

Answers to these questions may help in designing scientific methods to postpone or prevent the onset of the aging process, and thus control aging. This would prolong the active and youthful period from age 20-40 to say 20–60 or more. This would greatly increase the active period in humans, besides giving them the satisfaction of being youthful longer. The objective is to ensure better health and not prolongation of the years lived. Prolongation of the youthful period is expected to postpone or defer the onset of 'old age' diseases like cardiovascular and cerebro-vascular diseases, cancer, arthritis, *etc.*, which set in largely in-between 40 and 50 when the persons are at the peak of their careers.

Approaches to the Study of the Problem of Aging

Studies are being conducted on organisms of different complexities including man and other mammals (rats and mice), reptiles, amphibia, fish, insects (*Drosophila* and cockroach), free living nematodes and protozoa to understand the cause of aging at various levels: organismal, cellular, physiological, biochemical and genetic. Also, cells in *in vitro* culture, particularly fibroblasts, are being used for studying aging of cells with the hope that it may give some clue to organismal aging. Each model is useful in getting some insight into the problem of aging, and has also certain drawbacks. For example, when certain chemicals are administered to whole organisms and then organs are taken out for study, it is not known whether the chemicals entered the organs to the same extent in all ages. The use of cells *in vitro* has the drawback that while the effects of chemicals on tissues taken from different ages are studied, the effects of other organs and the components of the circulating fluid on the cells *in vivo* are ignored.

Theories of Aging

During the past two decades several theories have been put forth to explain the cause of aging. These theories fall into two major categories.

(a) Those that consider aging to be due to changes in various gene products like enzymes, collagen, hormones and immunoglobulins, and deterioration of subcellular structures such as cell membranes, lysosomes, mitochondria, or changes in homeostasis, defense mechanism, *etc.* It is obvious that such changes are secondary in nature and may have been due to certain changes preceding them. For example, the changes in the membrane structure would be due to changes in its composition such as lipids, largely phospholipids, and structural proteins. Lipids are synthesised by enzymes. If the lipid concentration decreases or varies, then it may be due to changes in the corresponding enzymes which synthesise them. The enzymes are coded by messenger RNAs which are the products of specific genes. If the level of an enzyme changes, it may be due to changes in its mRNA level which, in turn, may be due to the changes in the expression of the corresponding gene. Hence, the primary cause of aging may lie at the level of the gene.

(b) Those that explain aging on the basis of changes occurring at the level of the genome, the primary site, where all information for the formation and function of an organism is stored. Observations such as: (a) fixed life span of all individuals within a species ; (b) more or less a similar pattern of decline of various functions of all organisms after a short period of reproduction ; (c) long life span of progeny of long-lived parents and short life span of progeny of short-lived parents, and (d) similar life span of identical twins, indicate that the cause of aging may have a genetic basis. However, factors like stress, nutrition, temperature and heredity may influence the aging process that may account for the variability in the rate of aging and life spans seen among individuals of a species. Three theories have been put forward that attempt to explain the basic cause of aging at the level of genes. They are Somatic mutation theory (Szilard, 1959), Error Theory (Orgel, 1963) and Gene regulation theory (Kanungo, 1975).

Somatic Mutation Theory

This theory states that mutations occur randomly and spontaneously, destroy genes and chromosomes in postmitotic cells during the life span of an organism, and gradually increase its mutation load. This decreases the production of functional proteins, the number of postmitotic cells and various functions of the organism (Szilard, 1959). Rats exposed to wholebody irradiation that was too low to produce acute syndrome, died earlier than unirradiated controls. It was, therefore, proposed that irradiation accelerates the aging process. Later, Curtis (1963) reported that there is an inverse relationship between the dose of radiation given to mice and their longevity, and chromosomal aberrations like chromosome breaks and sister chromatid separation are greater in mice exposed to higher radiation.

These conclusions are, however, not supported by experimental data. The symptoms of senescence manifested in irradiated mice are different from those of normal mice. Chemical mutagens like nitrogen mustard do not give a dose-effect relationship as that for radiation. Both diploid and haploid males of the moth, *Habrobracon*, have the same life span. If mutagenic effect of radiation is the cause of aging, then diploids should have longer life span as they have double the complement of genes that should withstand radiation effect better. In analogous experiments, Holliday and his co-workers found that a certain proportion of human foetal fibroblasts in culture become tetraploid if they are treated by colchicine. However, both the tetraploids and normal diploids have the same division potential and life span. One of the difficulties of testing this theory is that there is no accurate and reliable method of measuring somatic mutations, and the symptoms developed in irradiated individuals are different from those of natural aging. Hence ionizing radiations and other mutagens may cause early death by increasing the frequency of cancer and other unknown derangements.

Error Theory

According to this theory (Orgel, 1963, 1973) errors occurring during information transfer processes like transcription and translation may cause accumulation of defective proteins and cause aging of cells and the organism. Such errors include incorporation of inappropiate nucleotides into messenger RNAs during transcription such that triplet codons are altered leading to incorporation of wrong amino acids into proteins during translation. Likewise, wrong amino acids may be picked up by tRNAs during translation and incorporated into polypeptides leading to amino acid substitutions and error containing proteins. If such proteins (enzymes) control metabolic paths, the defect may be short-lived and not deleterious as their effects would be erased as soon as they are degraded. However, if such errors occur in proteins responsible for protien synthesis such as RNA polymerase and aminoacyl-tRNA synthetases, then the effect would be disastrous for the cell as all types of mRNAs and/or enzymes synthesised would have errors. The error containing proteins would get amplified until the cell dies of 'error catastrophe'.

Fibroblast cells in culture have been used to test this theory (Holliday & Tarrant, 1972). It is suggested that cells in culture are potentially immortal and are initially uncommitted. However, after a certain period of population doublings, a few cells get irreversibly comitted to senescence and death. They multiply for a period, and after a certain number of divisions, called incubation period, they senesce and die. The uncommitted cells continue to divide. It is suggested that commitment of cells in culture may be due to errors and that somatic cells have less ability to regulate errors. It is argued that error is inherent in all processes of macromolecular information transfer and that "to sustain the prospect of further evolutionary change and to improve its chance of ultimate survival, an organism must make occasional copying errors". It is accepted that had the fidelity of information transfer system been absolute, there would have been no evolution. However, Hoffman has suggested that the translation machinery is such that errors are not possible and hence 'error catastrophe' cannot occur. Hopfield has suggested that errors are avoided in replication machinery due to "proof reading" and destruction of erroneous products. Kirkwood has suggested that these mechanisms may be switched off at a later stage of development.

Several workers, however, have failed to detect faulty proteins in old age. Studies by Kanungo and his co-workers (1980) on the kinetics, antigenic properties and peptide maps of several enzymes purified from old rats show that they are similar to those of the young. Gershon and his co-workers have shown that the antigenicity, Km, Ki and electrophoretic mobility of superoxide dismutase purified from young and old rats are the same. Isoelectrofocussing also shows no differences in their electric charges. However, differences in their specific activity and temperature sensitivity are seen. These differences are attributed to post-translational modifications of the protein. Similar observations were made for several enzymes of the free living nematode, *Turbatrix aceti*. Thus there seems to be no experimental proof to support the 'error' theory.

Gene Regulation Theory

Observations such as those mentioned under theories of aging (b) show that the basic cause of aging lies at the level of the gene. Two possible types of changes are possible at the level of genes during aging: (1) Genes may undergo structural changes resulting in the synthesis of wrong or inactive enzymes (proteins) that may cause deterioration of function of the organism; (2) the degree of expression of genes may change with age causing alterations in the levels of enzymes and deterioration of function.

In-depth studies carried out by Kanungo (1980) and his co workers have shown that (a) the levels of several enzymes decrease with age; (b) this decrease is reversed by steroid hormones which produce their effects at the level of genes and alter gene expression; (c) isoenzymes of several enzymes get expressed and repressed in a sequential manner during the life span; (d) the peptide maps of proteins and their kinetic and antigenic properties are the same for young and old animals. Since the primary structures of proteins do not change with age, but only their levels change, this must be due to changes in the expression of corresponding genes as a function of age.

Kanungo and his co-workers have further shown that (a) certain covalent modifications of histones such as acetylation and phosphorylation decrease with age. Since histones constitute an important constituent of the chromatin, such alterations may decrease gene expression ; (b) Digestion of chromatin by the endonucleases, DNase I (that cuts chromatin DNA at 10 base pair intervals) and micrococcal nuclease (MNase; that cuts DNA at 200 bp intervals) shows that the extent of digestion, particularly by DNase I, decreases with age. This indicates greater compaction of chromatin with increasing age. Such changes in chromatin may be modulated by hormones and other factors. The levels of these factors change with age. This may cause gradual alterations in gene expression and set in motion a deteriorating process leading to senescence.

These findings show that in the post-mitotic cells such as neurons, and skeletal and heart muscle cells in which the age-related functional decline is more pronounced, factors such as steroid and peptide hormones and other effectors that are produced at specific phases of the life span during growth and reproductive maturation, may bring about gradual alterations in chromatin conformation. This may change the expression of genes leading to alterations in the levels of enzymes and deterioration of functions. This supports the model for aging proposed by Kanungo (1975) according to which the regulatory mechanisms necessary for the normal functioning of the array of genes required for various functions including reproduction during adulthood gradually get destabilized as the organism is unable to maintain the levels of factors that are necessary for induction/repression of these genes. As a result of reproduction and other functions associated with growth, certain factors get depleted which the organism is unable to replenish, and certain factors accumulate which it is unable to get rid of. Hence, there is a gradual decline in the homeostatic functioning of the genes required for the maintenance of adulthood that leads to deterioration of function. Also, as a result of accumulation of certain factors and depletion of others, certain undesirable and harmful genes get expressed. This may result in the occurrence of cancer and autoimmune diseases whose frequency is known to increase with increasing age.

Some Important Biochemical Changes During Aging

Chromatin

All information in an organism is stored in DNA which is complexed with histones and non histone chromosomal (NHC) proteins to constitute the genetic apparatus called the chromatin. Histones are small molecular weight basic proteins being rich it lysine and/or arginine residues which are clustered in -NH_2 and -COOH terminal regions. There are five major types of histones, Hl, H2A, H2B, H3 and H4. They are synthesized during the Sphase of the cell cycle. Being basic, they bind to DNA and take part in the structural organization of chromatin. The fundamental structure of chromatin consists of a string of nucleosomes of ∾ 10nm diameter which have an internal core consisting of an octamer comprising two molecules each of H2A, H2B, H3 and H4 around which the DNA is wrapped. Hl is bound to the linker DNA that connects two nucleosomes and is involved in the higher order structure of chromatin. NHC proteins are highly heterogeneous, several hundred in number, rich in acidic amino acids, tissue and species specific. Tissues which are metabolically more active have more types and amounts of NHC proteins. They are synthesized throughout the cell cycle. NHC proteins are involved both in the structural organization of the chromatin and in the regulation of gene expression.

Both histones and NHC proteins undergo several types of post-synthetic covalent modifications — Phosphorylation, acetylation, ADP–ribosylation and methylation. Whereas the first three modifications decrease the net positive charge on histones and cause their dissociation from DNA, methylation strengthens their binding to DNA. These modifications occur in specific histones and at specific phases of the cell cycle and development of the organism. Such modifications may alter the structural organization of chromatin and also alter its template activity. Phosphorylation has been implicated in cell division, acetylation and transcription. Methylation and ADP-ribosylation may be involved in differentiation of cells. Several endogenous effectors like hormones and metal ions modulate these modifications, and thereby may modulate the template activity of chromatin and hence the expression of genes.

Structural and conformational changes occur in chromatin as a function of age. Its melting temperature (Tm) increases and extractability of chromosomal proteins decreases indicating stronger binding of the proteins to DNA. Also, the extent of covalent modifications of the proteins decreases, as also the modulatory effects of effectors such as 17β-estradiol and Na-butyrate on these modifications. Such changes may be due to conformational changes occurring in old age. Consistent with these, changes transcriptional activity of the chromatin decreases in several tissues as a function of age. These studies clearly show that structural changes in chromatin, especially in post-mitotic tissues in which it does not turnover, cause impairment of its template activity and also its response to modulators. This may cause a gradual decline in various functions of the organism and lead to senescence.

Changes in Enzymes During Aging

Various aspects of enzymes like their activities, isoenzyme patterns, induction and kinetic parameters have been studied as a function of age of different animals. The activities of certain enzymes expressed both as units per mg protein and units per mg DNA decrease, whereas those of a few others increase in old age. A few enzymes do not show any change with age. No specific class of enzymes shows a specific pattern of change, nor do all the enzymes of an organ or a cell organelle undergo a specific type of change. Since each metabolic path has enzymes which belong to various classes, it may be of value if all the enzymes of a path are studied under similar conditions.

Studies on alterations of isoenzyme patterns of various enzymes have given useful information about the types of changes that may occur at the level of the genome which may be responsible not only for the shifts in isoenzymes, but also in enzyme activities. Such shifts in isoenzymes may impair the subtle and fine control of metabolic paths because of the differences in their affinities for substrates and effectors. This may cause significant alterations in the activities of metabolic paths and result in functional changes of the organism. The shift towards H4-LDH in the brain, heart and skeletal muscle that occurs in old rats may make these tissues more aerobic. A shift towards cAAT-B in the liver of the rat in old age likewise may cause changes in the amino acid metabolism. Increase in these isoenzymes indicate an increase in the expression of the corresponding genes. Such changes in gene function in old age may be brought about by factors or effectors appearing/disappearing in old age.

Studies on induction of enzymes, especially by steroid hormones, show that in general, there is a decrease in the degree of induction in old age. In a few cases it has been possible to revert such decreases by administration of appropriate levels of hormones. It appears that the impairment of induction of AChE by 17β-estradiol that occurs in old age may be due to a decrease in the level of the estradiol-specific receptor. Also, in tissues like the liver, it may be possible to repair the damage to induction by stimulating the regeneration of the tissue. These studies show that the changes in enzyme activities, and their isoenzyme patterns that occur in old age are reversible, and it may be possible to revert them to the adult levels.

Studies on kinetic and molecular properties of certain enzymes show that several properties like Km, Ki, mol. wt and electrophoretic mobility of enzymes of young animals are similar to those of the old. However, the specific activities of several enzymes studied so far decrease in old age, and their thermal sensitivity increases. These may be due to post-translational modifications. Hence, it appears that the same gene is responsible for the synthesis of an enzyme throughout the life span. These studies in conjunction with those on isoenzymes and induction give strong evidence in favour of the view that the changes in the levels of enzymes seen in old age are due to regulatory changes in the activities of corresponding genes that may be brought about by factors that may appear/disappear after adulthood. Such changes may cause deterioration of function, or decrease in the adaptability of the organism to endogenous and exogenous factors.

Changes in Collagen

Collagen is a structural protein. It is the most abundant protein in the body and is easily extractable. Hence several workers have studied its molecular properties as a function of age. Collagen consists of fibrils which are made up of collagen monomers. Each monomer is a triple helix of three poylpeptide chains. It shows molecular polymorphism because of the occurrence of two types of chains, α_1 and α_2. There are four variants of α_1. Each type of chain is coded by a specific gene. Four major types of collagen are found in tissues depending on the types of chains that form the collagen triple helix: types I, II, III and IV. Different types and proportions of chains are synthesized in different tissues. Their proportions change during development of the animal.

The pro-α chains are synthesized in the polysome. The prolyl and lysyl residues are hydroxylated by respective hydroxylases during and after chain elongation. Glycosyl transferases glycosylate hydroxylysyl residues in the cell. The assembly of three pro-α chains occurs in the rough endoplasmic reticulum to produce triple helical pro-collagen monomers which are then secreted to the extracellular matrix. Two separate pro-collagen peptidases remove propeptide fragments from the $-NH_2$ and $-COOH$ terminals. Lysyl oxidase oxidatively deaminates lysyl residues to produce allysines which crosslink with lysyl or allysine residues in the α chains of the same or other monomers and form collagen fibrils. The levels of enzymes responsible for the above modifications change during the life span of animals. Thus the age-related structural changes in collagen appear to be due to alterations in the activities of enzymes necessary for their structural modifications, and hence are secondary causes of aging.

Changes in Hormonal Function

Hormones regulate functions at various levels of organization. They play important functions during development and are responsible for conferring reproductive ability to the animal. The effects of lipid soluble hormones, the steroids, are produced through specific cytosol receptors at the level of the gene. The actions of peptide hormones are, in general, brought about through receptors present on the cell membrane. Steroid hormones, particularly the sex steroids, decrease in level in old age. Such a general decrease is not seen for peptide hormones. Also the receptors for certain steroids, decrease in old age as do the various responses of tissues to these hormones. No specific pattern of alteration in the level of peptide hormone receptors and responses of tissues to these hormones occurs as a function of age.

Induction of several enzymes by steroids hormones is impaired in old age. The impairment may be in the time required for induction or the magnitude of induction. The changes with age in the levels of hormones and the responses of tissues to hormones, however, do not give any insight into the basic cause of aging, as such changes may be due to impairment at any one or more sites and hence are secondary in nature.

Changes in Immunological System

The immune system is responsible for the protection of the body against various types of diseases that are caused by pathogens. The frequency of infectious and auto immune diseases, and cancer increases after adulthood. The immunocompetence of animals and humans decreases after adulthood. Hence it is possible that the deterioration of the immune system and the consequent onset of diseases may be responsible for the decline in vigour after adulthood.

The immunological responses of animals to various foreign substances including viruses, bacteria and foreign cells are due to two major types of lymphocytes, B and T cells. B cells are processed through the bursa of Fabricius in birds and through the bone marrow in mammals, and produce specific antibody or immunoglobulin (Ig) when provoked by a specific antigen (Ag). The Ig inactivates the Ag. The B cell function depends on cooperation from T cells. Studies so far show that the B cell function apparently does not deteriorate much with age (Makinodan, 1977).

T cells are processed through the thymus. Three major types of T cells are known: helper, suppressor and killer. Helper T cells are necessary for Ig production by B cells. Suppressor T cells suppress the production of Ig, and may be responsible for self tolerance by preventing lymphocytes from reacting against the host's own cells. The killer T cells directly react with antigens and inactivate them. Several studies, both *in vivo* and *in vitro*, have shown that the functions and nunmber of various types of T cells, particularly of suppressor T cells, decline after attainment of reproductive maturity. Several functions of T cells appear to be controlled by a complex set of genes, the major histocompatibility complex (MHC) system. Hence deterioration of T cell function may be due to alterations in the genes in the MHC.

Even though the cause of decline of immune system remains to be elucidated, attempts have been made to promote the immunofunction of old animals by transplantation of immune cells from young donors. Such methods may delay the onset of diseases for certain periods and possibly extend the life span.

Cellular Aging

Both unicellular and multicellular organisms age. When individual cells especially fibroblast cells, are taken out of the latter and kept in a culture medium, they divide for a definite number of times and then senesce and die. So aging of multicellular organisms may be due to the aging of individual cells. Furthermore, cells of all types undergo aging and lose their proliferative activity after a certain period *in vivo*, *in vitro* and even when transplanted to younger hosts. This property is apparently not due to any cytoplasmic factor, and may be determined by the genome. Hence, cellular senescence and death are intrinsic properties of cells. The proliferative capacities of different cell types are different, and this may depend on the type and degree of their differentiation. Some cells stop dividing at an early stage of development, and some others continue to divide throughout the life span.

The cells of the skin, haemopoietic system and mammary epithelium which retain growth and proliferative activities till the end of the life span can be rescued from senescence and made to remain viable for periods far in excess of their maximum life span if they are serially transplanted in younger hosts. Thus the growth and viability of cells can be altered by suitable manipulations. Also, these properties appear to be determined by the physiological status of the animal and not by its chronological age.

Free Radicals and Age Pigment

Free radicals are produced during oxidation reactions in cells. They are highly reactive as they have odd number of electrons. Some of the common free radicals are O_2^-, HO^- and ROO^-. They cross-link biomolecules such as enzymes and nucleic acids and inactivate them (Harman, 1962). They cause peroxidation of unsaturated fatty acids and thus destroy biological membranes. Fortunately, the free radical, O_2^- (super oxide), is neutralised as soon as it is formed due to the presence of superoxide dismutase (SOD) which dismutates or rearranges two molecules of O_2^- to produce O_2. It has been shown that the level of SOD decreases with age (Reiss & Gershon, 1976) which may be the reason why there is a possibility of an increase in free radicals with age that may inactivate enzymes and decrease overall function.

Age pigment (lipofuscin) occurs generally in the cytoplasm of post-mitotic cells such as neurons, cardiac and skeletal muscle cells (Strehler, 1951). Its amount increases with age. It is a highly complex, conjugated, cross-linked material that accumulates largely in post-mitotic and aerobic cells. It is speculated that it arises by peroxidation of polyunsaturated lipids in cellular organelles like mitochondria and lysosomes. Accumulation of a large amount of a inert pigment in the cytoplasm may inactivate cell function. Attempts have been made to eliminate or prevent the accumulation of age pigment in tissues by administration of drugs such as centrophenoxine and dimethylaminoethanol (Nandy & Bourne, 1966; Hasan *et al.*, 1974; Roy *et al.* 1983). These drugs are reported to improve the functioning of organs. It is obvious that age pigment is a secondary cause of aging. The basic cause of formation of age pigment is not known yet, and until then, no appropriate method can be divised to prevent its accumulation.

The rapid increase in the proportion of old people in all countries including India is a matter of great concern for all Governments. Discovery of the basic cause of aging may help in divising methods to postpone the onset of old age and thereby alleviate the old age problems to a great extent. The department of Science and Technology, Government of India, has recognised the problem of aging as a thrust area of research. Greater involement of scientists from diverse fields and larger amount of funds would go a long way in contributing towards this goal.

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5

COLOUR PHYSIOLOGY OF CRUSTACEA

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Introduction

Colour changes in animals have commanded the attention of investigators since Aristotle, but interest in this subject has never been higher than it is at present. Colour changes in crustaceans are accomplished by means of specialized cells called as chromatophores, which can disperse or concentrate the pigment within them, thereby changing the body colour of the animals. The colour changes in crustaceans were first recorded by Kroyer[1] in the prown, *Hippolyte*. Evidence revealing that chromatophores of crustaceans are hormonally controlled was obtained in 1920's based on the experiments involving blood transfusion with the shrimp, Crangon[2,3]; and removal of the eyestalks from the prawn, *Palaemonetes*, which resulted in permanent dispersion of red pigment in the chromatophores[4]. Injection of eyestalk extract caused a rapid transitory blanching. Brown[5] concluded that each of the chromatophoric pigment in the prawn, *P. vulgaris*, was under separate hormonal control.

In 1948, Parker published a comprehensive volume on the control of chromatophores. Since the publication of Parker's volume, several reviews of the literature concerning colour changes have appeared[6–16].

Fingerman[17] reviewed the literature on chromatophores and colour changes in the invertebrates as well as vertebrates.

The research on colour physiology, in the 1970's was directed towards (1) the relationship between neurosecretory granules in arthropods and circulating forms of the various chromatophorotropins and (2) the chemistry of the arthropod chromactivators.

The neuroendocrine sources of crustacean chromatophorotropins have been extracted in a variety of solovents, especially distilled water, saline and ethanol [18,19]. Noel[20] demonstrated a neural control of chromatophores in the shrimp, *Processa edulis*, and observed blanching of half of the body from the head to tail after unilateral eyestalk ablation.

Eventhough substantial evidences is available for nueroendocrine regulation of crustacean chromatophores, the studies of Aoto[21], Burgers[22] and Bauchan and Mengoet[23] indicated that in addition to the well established peptide neurosecretory products some indolealkylamines may also be involved in the chromatic physiology of crustaceans. It has been observed that biogenic amines like serotonin, epinephrine caused pigment dispersion in the erythrophores of prawn, *Palaemon squilla*[24]. The studies of Murdock[25], Gerschenfeld[26] and Kerkut[27] on the presence of neurotransmitters in the crustaceans have given some clue to carry out further work on the role of neurotransmitters in the chromatic physiology of crustaceans.

A survey of the literature shows that alkaloids like reserpine, caffeine, theophylline and colchicine have some effect on colour changes mechanism either directly or indirectly or via some other mechanism. The effect of alkaloid caffeine, on the chromatophores has been studied in *Carcinus maenas*[28] and *Caridina rajadhari*[29]. Theophylline is a central nervous stimulant and it's effect on the migration of the pigment in chromatophores is studied by Noel[20] in the shrimp, *Processa edulis*; and Kandharkar[29] in the prawn, *Caridina rajadhari.* Colchicine is known to disrupt the microtubules presumably by shifting their equilibrium towards tubulin[30]. It is also known to inhibit pigment concentration in the crustaceans[31-34]. Only few studies have been made on the effect of reserpine on the crustacean colour changes[35,29].

Types of Chromatophores

Cells containing pigment that can disperse or concentrate, thereby changing the tint of the organism in which they lie, are known as chromatophores. Such effectors may be close to the surface of an organisms of deep surrounding various organs.

Chromatophores in crustaceans are uninucleate cells, usually multibranched and in which the pigment was maximally concentrated. The shape of the chromatophores is unchanged as its contained pigment migrates from the fully dispersed condition to the maximally concentrated state and back again to full dispersion. Chromatophores are highly differentiated for specific synthesis or accumulation of pigment of well defined chemical nature such as carotenoid, ammochrome, pteridine, flavin, melanin *etc*. In some animal groups, unicellular or groups of unicellular chromatophores are so intimately arranged that they appear as a single unit. Such colour units have been termed as chromatosomes.

Chromatophores are classified according to the pigment or pigments contained therein. If the pigment is brown or black, the cells are referred to as melanophores. Yellow chromatophores are called *Xanthophores*; red ones, *erythrophores*. Cells that contain guanine or guanine like substances are called *guanophores*. When the guanine consists of fine granules that can migrate, the term *leucophore* is usually employed. In some cells, called *iridiophores* or *iridiocytes*, the white pigment consists of plate-like crystals that give the bearer an iridescent sheen. Iridiophores are technically not chromatophores according to the definition supplied above, because the pigment in these cells does not migrate. The term *lipophore* has been used as a general term for chromatophores in which the pigment is soluble in organic solvents. Yellow and red pigments fall into this category.

Crustacean chromatophores may contain one (monochromatic) two (dichromatic) or serveral (polychromatic) pigments. Some chromatophores are multinucleate, and seen to be groups of anastomosing cells. Polychromatic chromatophores are characteristic of the prawns and shrimps (Decapoda, Natantia); Mono and dichromatic chromatophores are found in the decapod Astacura, Isopoda, Stomatopoda, Anomura and Brachyura.

Structure of Chromatophores.

It was beleived earlier that highly branched, unicellular chromatophores are amoeboid, sending out pseudopodia-like processes when pigment dispersion occurs. It is now generally accepted that they have a fixed cell outline and that pigment migrates into and out of fixed processes[36],[37] (Fig. 1). This view is supported by the electron microscope observations of melanophores in the teleost, *Labistes reticulatus*[38]. Each melanophore has thick cell membrane of fixed position, which measures about 1200 Å, followed by a thin membrane of about 80 Å, which contains the pigment granules, nucleus and mitochondria. Between thick and thin membrane lies the zone of fibrils, which are not attached to either of the membranes. When these fibrils contract, the inner sac gets squeezed and pigment concentration occurs. On the other hand, dispersion of the pigment occurs by relaxation of the fibrils which in turn allow the sac to expand. In crustaceans, unicellular chromatophores are highly branched (Fig. 1). A pigment within each chromatophore either disperses or concentrates to alter its shade or tint. When dark pigment is dispersed through each of many chromatophores, an overall dark coloration of the animal results; when the pigment is concentrated the animal appears beanched.

Classification of Chromatophore Responses

Colour changes have been divided into two categories, morphological and physiological. The morphological colour change involves a change in the quantity of pigment within the existing chromatophores or a change in their number. It has been shown that these changes involve chemical alteration in the pigments and are not merely a translocation of pigments within the body[39]. Morphological colour changes can occur in a number of ways: (1) by an increase (or decrease) in the number of chromatophores per unit area of body surface[40]; (2) by an increase (or decrease) in pigment concentration[39,41]; and (3) by a combination of these two.

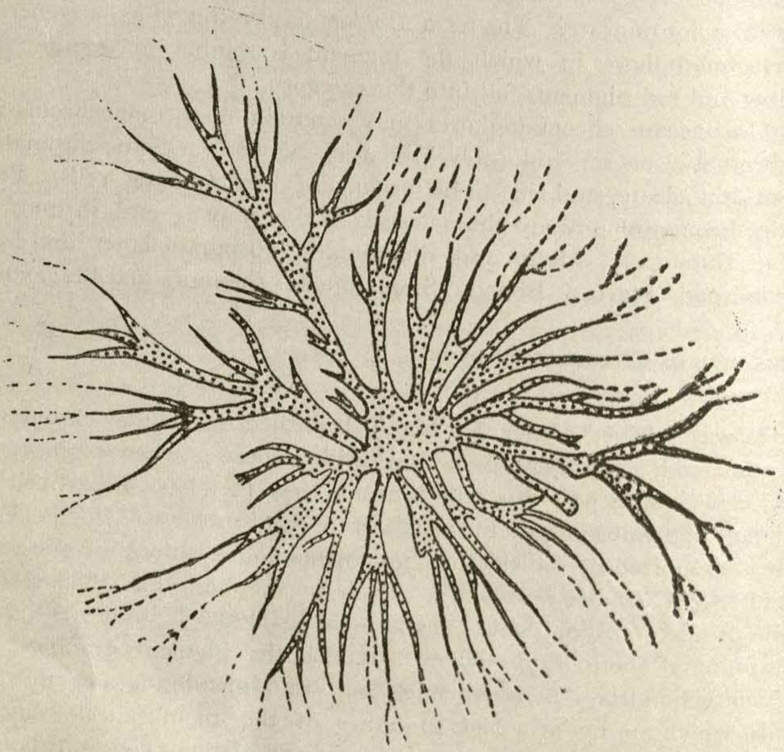

Fig. 1 Diagram of a chromatophore from the uropod of the prawn, *Leander serratus*; the pigment is shown in the expanded state.

The physiological colour change is brought about by movements of pigment granules within the chromatophores. This was first studied by Pouchet[42]-[44], and was placed on a firm experimental basis by Keeble and Gamble[45]. Physiological colour changes are ordinarily more rapid and can be evoked by any of the several factors such as light, temperature humidity, endogenous rhythms, psychic stimuli and tactile stimuli.

Responses of chromatophores to light may be divided into two categories, primary and secondary. Primary colour changes typically occur through routes other than the eyes *i.e.* by direct action or light on the chromatophore, the more common type, or through an extra ocular reflex, a response that involves either nervous or endocrine co-ordination between a stimulus received by a receptor other than the eye and the chromatophore. Primary response, although typically associated with larval or embryonic pigment cells that become fully functional before the eyes are operative, may be exhibited by chromatophores of adults. Secondary responses depend upon the nature of the background and not on the intensity of light. The degree of pigment dispersion is determined by the ratio of the amount of light directly falling (incident) on the eye to the quantity impinging on the eye after reflection from the background. This ratio is known as the "albedo". In most adults the primary response is dominated by the secondary one.

Chemistry of Chromatophoral Pigments

The chemistry of chromatophoral pigments has been discussed by Goodwin[46] for crustaceans. The pigment in the melanophores of brachyuran decapods is melanin. This pigment is brown or black and can be extracted from tissues with boiling alkali. Chemically, two melanins are probably polymerized indole quinones formed by the action of tyrosinase on aromatic aminoacids, especially tyrosine. The dark pigments in crustaceans aside from the crabs appear to be ammochromes rather than melanins. The red and yellow chromatophoral pigments are carotenoids. The crustaceans obtain carotenoids in their diet but can alter them by oxidation. Astaxanthin is probaly the most common chromatophoral carotenoid in crustaceans[38].

Functional Significance of Colour Changes.

The commonly ascribed function of colour changes are (1) protective colouration; (2) thermoregulation and (3) displays associated with mating and parental behaviour. Dispersion of dark pigments, when an organism is put on a dark background, obviously adapts an organism better for both aggression and survival. Concomitant with the ability to change colouration for protection of the individual should be the ability to select the background that would assure maximum protection. Brown[47] showed that the cryfish, *Orconectes immunis*, has the ability to select between black and white backgrounds, always tending to select black over white.

Primary responses of chrotophores to light have been noticed in a wide variety of invertebrates. Quite often these responses are intimately associated with responses to temperature that have been interpreted as thermoregulatory. Brown and Sandeen[48] determined the responses of the black and white chromatophores of the fiddler crab, *Uca pugilator*, to light and temperature. Both pigments dispersed with increased illumination. These pigments also showed a temperature response. The black pigment tended to concentrate as the temperature was raised above or lowered below about 15°C. White pigment, on the other hand, tended to disperse as the temperature increased above or decreased below about 20°C. The responses to high illumination and temperature may be basically thermoregulatory for protection of the protoplasm of the crabs. The optimal condition of the chromatophore system to produce dissipation of light and heat when the temperature is high would be, as is the actual case, dispersion of white pigment and concentration of black. Concentration of the black pigment tends to diminish the surface area that absorbs light, but dispersion of the white pigment tends to increase the area that reflects it.

Melanin of the blue crab, *Callinectes sapidus*, also responds to illumination. Between 12 and 3000 ft. c. the melanin became more dispersed[49]. As the temperature increased from 10 - 28°C the melanin of *Callinectes* concentrated. The latter response can be interpreted as thermoregulatory. The tendency of melanin to concentrate with increased temperature, thereby reducing the light-absorbing area, may be a primitive attempt at homoiothermis.

Colour changes associated with mating and parental behaviour have not received much attention.

Staging of Chromatophores.

The shade of the body colour depends on (1) the number of chromatophores, (2) the nature of the pigment each chromatophore contains, and (3) the degree of dispersion of the contained pigment. Early workers simply described the shade of an organism in macroscopic terms such as dark, intermediate or light. For quantitative work, several methods have been discribed by Parker[50]. The most popular is that of Hogben and Slome[51], who divided the entire range from maximum concentration to maximum dispersion into five stages (Fig. 2). According to their scheme, stage 1 represents the most concentrated condition of the pigment, stage 5 the most dispersed, and stages 2, 3 and 4 the intermediate conditions. These five stages closely correspond to the descriptive stage of (1) punctate, (2) punctate stellate (3) stellate, (4) stellate-reticulate and (5) reticulate. The obivious advantages of this system are that first it allows direct, accurate observation of individual chromatophores and second, it facilitates quantitative study and graphic representation of the changes of the degree of pigment dispersion. The major criticism of the Hogben and slome method are that (1) the

FIG-2

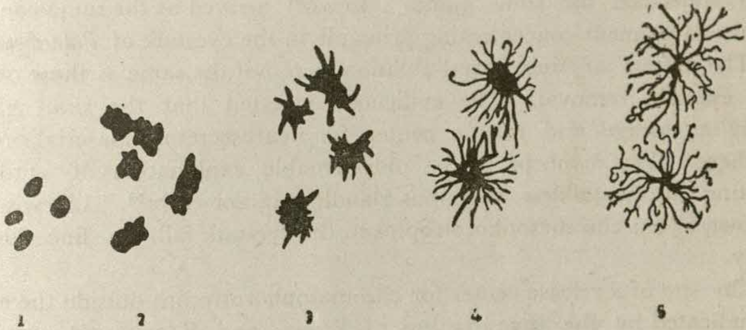

Fig. 2 Chromatophore stages of Hogben and Slome (1931).
Stage 1 ; Pigment fully contracted,
Stage 2,3 and 4 : Progressive pigment expansion
Stage 5 : pigment fully expanded.

method is subjective and (2) the stages may have no mathematical significance and might be constucted as indicating, for example, that stage 4 represents twice as much pigment dispersion as stage 2.

The photoelectric method of Hill *et al.*[52], measurement of the fraction of incident light reflected from a unit area of skin surface, was devised to eliminate the subjective aspect of the method of Hogben and Slome. The obvious defect in the photoelectric method is that the amount of reflected light depends on both the degree of pigment dispersion and the number of chromatophores. Furthermore, in the same piece of skin one pigment may disperse and another concentrate, but the photoelectric method reveals only the net change in the light reflected by the pigmented surface and not the changes in the individual chromatophores. Thing[53] discussed the difficulties inherent in both the techniques. As a result of his experiments, he concluded that the results obtained by the two methods are nearly the same and both the methods are equally suitable for assays He preferred the Hogben and Slome method because of its inherent simplicity.

Sources of Crustacean Chromatophorotropins

After the initial discoveries of Perkins[4] and Koller[54] that chromatophorotropins are present in the eystalk of crustaceans, systematic studies were undertaken to locate the sources of these hormones. Hanstrom[55] found two structures in the eystalk, the sinus gland and X-organ, which he suspected of having endocrine activity.

The sinus gland was found to contain a greater concentration of chromatophorotropins than any other structure in the eyestalk. Carlson[56] found that the vast majority of melanin dispersing hormone in the eyestalks of the fiddler crab, *Uca pugilator*, was located in the portion of the eyestalk which contained the sinus gland. Brown[57] arrived at the same conclusion for the red pigment concentrating principle in the eyestalk of *Palaemonetes*.

The effects of sinus gland ablation were not the same as those observed after eyestalk removal. The evidence suggested that the sinus gland is merely a storage and release center for neurosecretory material produced elsewhere. This concept is the only tenable explanation of studies of moulting in eyestalkless and sinus glandless fiddler crabs[58]. Likewise, what is known about chromatophorotropins in the eyestalk falls in line with this theory.

On site of a release center for chromatophorotropins outside the eyestalk was indicated by the investigation of Brown and Ederstron[59] who found that the portion of the circumoesophageal connective just posterior to the connective ganglia contained more melanin dispersing hormone for *Crangon septemspinosus* than other central nervous organs outside the eyestalk. Brown[60] later found that the tritocerebral commissure which runs posteriorly from the connective ganglion on one side down the medial surface of the connective for a short distance and then proceeds to the centrolateral connective around the posterior side of the oesophagus together with its immediate continuation within the circumoesophageal connectives, contained all of the *Crangon* darkening hormone occurring within the central nervous system. Brown, Webb and Sandeen[61] found that the tritocerebral commissure was a potent source of red pigment concentrating hormone in the prawn, *Palaemonetes vulgaris*. The tritocerebral commissure contained as much of this hormone as did one sinus gland.

In *Palaemon serratus* no discrete sinusplate was seen, but the post commissure nerves were wide and flat for the proximal part of their course; many acidophilic droplets lay in the flat portion of the post-commissure nerves and in the epineurium of the commissure nearby. The droplets lay along definite pathways which appeared to radiate from the bases of the post-commissure nerves. Large cells suspected to be secretory were found within the commissure, close to its hinder margin near the points of emergence of the post commissure nerves and in the connective ganglia. Injection experiments revealed that those regions richest in acidophilic droplets yielded extracts that contained large quantities of red and white pigment concentrating principles. Knowles suggested that these chromatophorotropins are produced by neurosecretory cells in the tritocerebral commissure and in the connective ganglia and are stored and released by specialized portions of the epineurium of the post-commissure nerves and of the commissure. These specialized portions have been termed the post-commissure organs[13].

One of the earliest pieces of evidence in support of the concept that chromatophorotropins occur in central nervous organs outside the eyestalk was the observation that stimulation of the eyestalk stubs of the prawn, *Palaemonetes*, results in transitory blanching whereas the fiddler crab, *Uca* darkens [41],[62]. These chromatophorotropins are presumably released from the post commissure organs which possess all the characteristics of neurohaemal organs.

Physiological evidence in favour of the hypothesis that chromatophorotropins occur within neurosecretory granules of crustaceans which are bounded by a simipermeable membrane was presented by Perex-Gonzalez[63].

Fingerman and Aoto[64] reported that removal of both eyestalks of the dwarf crayfish resulted in accumulation of neurosecretory material in the supraoesophageal ganglia and in the stubs of the optic nerves. These stubs hypertrophied and became engorged with neurosecretory droplets. A portion of the accumulated material proved to be red pigment concentrating hormone. These observations were consistent with the hypothesis that neurosecretory material produced outside the eyestalk is transported into the eyestalk via axons in the optic nerve.

Chemical Nature of Crustacean Chromatophorotropins

Carlson[65] was the first investigator who attempted to determine the chemical properties of eyestalk extracts. He found that the melanin dispersing hormone of *Uca pugilator* would readily diffuse through a cellophane membrane. This hormone is soluble in ethyl alcohol but insoluble in ether and can be boiled for a few minutes in dilute HCl or NaOH without loss of activity. These results show the hormone has a relatively low molecular weight, is heat stable and is not a lipid.

Experiments performed in the Fifties led Knowles and Carlisle[10] to postulate that crustacean chromatophorotropins are peptides. The results of subsequent investigations lend support to this hypothesis. The red pigment concentrating hormone in the post commissure organs of *Palaemon serratus* is destroyed by trypsin and by acid hydrolysis, indicative of peptide bonds[66] and not inactivated by amine oxidase or by orthodiphenolexidase, thereby suggesting that the red pigment concentrating substance is not a catechol amine[13].

Fingerman[14], using trypsin, incubated extracts of eyestalks and supraoesophageal ganglion plus circumoesophageal connectives of *Cambarellus shufeldti* and found almost complete inactivation of the red pigment concentrating and red pigment dispersing hormones. In spite of the growing feeling that chromatophorotropins of crustaceans are polypeptides, one must be cautioned that much of this sentiment is based on inactivation by proteolytic enzymes whereas trypsin and chymotrypsin, for example, also have a weak esterase activity and inactivation by these enzymes is in itself insufficient proof of the peptide nature of these chromatophorotropins. Fingerman and Aoto[71] and Fingerman and Mobberly[72] studied the electrophoretic behaviour of the red pigment dispersing substances at different pH values. The data lend support to the hypothesis that crustacean chromatophorotropins are peptides because of the charge reversal these substances showed at different pH values.

The structure of the red pigment concentrating hormone in the eyestalks of *Pandalus borealis* has been determined and then confirmed by synthesis from amino acids[67]. Melanin dispersing in the eyestalks of *Uca pugilator* can be extracted by saline or distilled water as polypeptide alone or by ethanol as a complex of polypeptide with a lipoprotein molecule[73].

Fernkind and Josefsson[67] characterized the red pigment concentrating hormone (RPCH) of *Pandalus*. They described the chemical synthesis and the amino acid sequence of this hormone. The structure and the amino acid sequence of this hormone is described as Glu-Leu-Asn-Phe-Ser-Pro-Gly-Tryp-NH_2.

A hormone with a similar cellular specificity seems to be released by dopamine in *Uca*[68]. The light adapting distal retinal pigment hormone was characterized from the eyestalks of *Pandalus borealis*[69]. The amino acid sequence of this hormone is Asn-Ser-Gly-Met-Ile-Asn-Ser-Ile-Leu-Gly-Ile-Pro-Arg-Val-Met-Thr-Glu-Ala-NH_2[70].

In the present review, sources of chromatophorotropins in some freshwater prawns and crabs studied from the Crustacean endocrinology laboratory, Marathwada University, Aurangabad are described. In the freshwater prawns, *Caridina weberi and C. rajadhari*, the neurosecretory cell groups were seen on medulla externa and interna of the eyestalks. The X–organs constitute a few clusters of neurosecretory cells lying on the ventral side of the eyestalk between the medulla terminalis and medulla interna and extending upto the peduncle lobus opticus. The sinus gland is situated in the dorso-distal part of the eyestalk between medulla interna and externa and is innervated from the medulla terminalis through the axons. Neurosecretory cell groups were also noticed in the supraoesophageal ganglion, thoracic ganglion and abdominal ganglia[18],[29]. The neurosecretory cells in *Macrobrachium kistnensis* were found to be distributed in groups in the eyestalks, supraoesophageal ganglion, tritocerebral commissural ganglia, thoracic ganglion and abdominal ganglia[19]. Diwan and Nagabhushanam[74] in the crab, *Barytelphusa cunicularis*, described three types of neurosecretory cells (A, B and C) present in groups in the eyestalk, supraoesophageal ganglion, commissural ganglia and thoracic ganglion.

The neurosecretory cells in all the decapods described above were large, having nucleus, abundant cytoplasm and droplets of granules in the perikaryon, which stained red with Mallory's, blue-black with CHP and Gomori's Stain.

Physiology of Colour Change

Diurnal Rhythm

Rhythm of colour change since it was intrigued by Abramowitz[75,76], attracted the attention of a number of investigators. Fingerman[37] reviewed extensively the rhythm of migration of pigments. The cyclic colour change is characterized by greater dispersion of the melanophoric pigment by day than by night in the brachyuran crustaceans[77,78]. However, very little work has been done on the circadian rhythm of colour change in the natantian decapods. Scheer[15] reported a diurnal rhythm of colour changes in the monochromatic erythrophores of *Palaemon xiphias*, *Processa acutirostres* and *P. edulis edulis*. The prawns remained pale and transparent in the day, while at night they were bright red in colour. However, Madhyastha and Rangnekar[79] have reported red pigment dispersion during the day and concentration at night in the marine prawn, *Metapenaeus monoceros*. Humbert[80] observed diurnal rhythm for colour change in the marine prawn, *Palaemon serratus*; this rhythm disappeared after extirpation of sinus glands.

In *Caridina weberi*, Nagabhushanam and Vasantha[81] described a diurnal rhythm of pigment migration for white and red chromatophores. On a neutral background, these chromatophores show full dispersion at noon and full concentration at midnight. Maximum dispersion of white pigment was observed to stage 4 in noon, whereas, red pigment dispersed to only stage 2.5. However, degree of concentration was the same in both the pigments reaching the punctate stage at midnight.

Keshavan[19] observed a diurnal rhythm for the dispersion and concentration of yellow chromatophores in the prawn, *Macrobrachium kistnensis*. The yellow chromatophores were dispersed by night and concentrated by noon, unlike the red and white chromatophores of *C. weberi*.[81]

In the fiddler crabs, most of the observations on the colour change have been pertained to the melanophores[82]. However, very little is known about the movement of red pigment in these crabs. In *Gelasimus annulipes*, Nagabhushanam[78] has demonstrated a circadian rhythm for the red pigment migration, which dispersed by day and concentrated by night. In a freshwater crab, *Barytelphusa cunicularis*, a similar diurnal rhythm for red and black chromatophores was observed, being more dispersed during day time and fully concentrated at night[83].

Fingerman[17,37] has shown that the daily rhythm of the melanin concentration and dispersion in fiddler crabs is unaffected by temperature changes in the range of 6–26°C The prawns, *C. weberi*[81] and *M. kistnensis*[19], when kept under black background or white background did not show daily rhythm of pigment migration. This circadian rhythm was only observed under the neutral background. In *Palaemon serratus*, Humbert[80] found disappearance of the rhythm for colour change after the removal of sinus gland. However, in the fiddler crabs, webb *et at.*[84] have shown that the eyestalkless crabs were more responsive by day than by night to an extract containing melanin dispersing hormone. The daily rhythm of melanin dispersion could be restored in eyestalkless individuals as the melanin of the eyestalkess crabs did become dispersed during the day, then blanched during the night and redispersed the following day. The melanin concentrating hormone in eyestalkless crabs is probably released from the postcommissural organ, the neurohaemal organ other than the sinus] glands of the eyestalks, which is known to contain chromatophorotropins.

Besides diurnal rhythm, Brown *et al.*[85] have also demonstrated a tidal rhythm of colour change. They have observed migration of melanin during the day time 1–3 hrs after the low tide in the chromatophores of the fiddler crabs living on a beach with simi-diurnal rhythm.

Background Adaptation

Background responses resulted in dispersion of dark pigments on a black background and concentration on a white background. The white chromatophores usually respond to backgrounds in a manner opposite to dark pigments. Background responses have been studied in several natantians. In brachyurans, background responses have been demonstrated, besides others in *Gelasimus annulipes*[86,78] and *Metapograpsus messor*[87].

In *Caridina weberi*, *Vasantha*[18] demonstrated that on a white background, white pigment became maximally dispersed and the red pigment maximally concentrated, whereas on a black background the condition was reversed. On white, green, blue and yellow backgrounds, the red pigment was completely concentrated, while on a black background it was fully dispersed and on a red background it was in an intermediate stage of dispersion. *Macrobrachium kistnensis*, when placed in different coloured background as in the case of *C. weberi* exhibited a maximum concentration of yellow pigment in green background. Whereas, on a yellow background they disperse fully. However, the yellow pigment reaches to an intermediate stage of dispersion in blue and red backgrounds[19].

In *Caridina weberi*[18], when placed in white and black backgrounds for two hours, the white pigment dispersed maximally and concentrated fully, respectively. Similar results were obtained with red pigment also. The red pigment dispersed fully on a black background and fully concentrated on a white background, if the prawns were kept in white and black backgrounds, respectively. With increase of time of exposure to background, the rates of pigment migration decreased progressively. A similar phenomenon was also noticed in the crayfishes, *Cambarellus*[88] and *Orconectes clypeatus*[71].

Brown and Ederstrom[59], while working on the shrimp, *Crangon septemspinosus*, have demonstrated that the shrimps are usually dark on a dark background and pale on a white background, but often some individuals remain dark on a white background and vice versa. Later, Chassard$_{86}$

explained that the shrimp, *Crangon crangon*, when placed alone in a white background remains blanched. However, when two or more specimens are placed together in a white container, their adaptation depends not upon the background but upon the colour of each other. When 10 dark specimens were placed into a white container, they all remained dark for atleast three days and when a pale specimen was placed among them, it soon darkened, although, the background was white.

Light

It is known that light and temperature influence pigment migration in the chromatophores of crustaceans. The responses of light can be divided into two categories: (1) primary and (2) secondary. In the former, black and white chromatophores are dispersed with increase of total illumination, while later typically results in dispersion of black pigment on a dark background. The black and white pigments of the fiddler crab, *Uca*, disperse with increased illumination. Nagabhushanam[78],[90] has shown in the shrimp, *Alpheus malabaricus* and crab, *Gelasimus annulipes* that red pigment disperses with increased illumination. Diwan[83] reported that the black and red chromatophores of the crab, *Barytelphusa cunicularis*, disperse with increased illumiantion.

In the prawn, *Caridina weberi*, Vasantha[18] observed dispersion of white pigment in white and black background with increase in the illumination, and this dispersion was always more on the white background than on the black background at all intensities. Keshavan[19] in *Macrobrachium kistnensis* has shown that the yellow chromatophores concentrated with the increased illumination. The results of Vasantha[18] and Keshavan[19] do not agree with the results of Diwan[83]. Dispersion of dark pigments with increased illumination was also found in the prawn, *Metapenaeus monoceros*[91], shrimp, *Alpheus malabaricus*[90], *Gelasinus annulipes*[78] and *Metapograpsus messor*[92]. The dispersion of pigments in response to high illumination may serve as a protective mechanism. The darkened surface prevents light from penetrating the deeper tissues, and hence protects the protoplasm.

Temperature

Effect of temperature on chromatophore system was first described by Jourdain[93] *in Nica edulis* wherein, the activity of colour change became sluggish at lower temperature (5–6°C). Matzdroff[94] reported that the chromatophores of *Idotea tricuspidata* did not respond to either low or high temperatures. Keeble and Gamble[95] demonstrated that the concentration of the dark pigment of *Hippolyte varinus* takes place above 32°C and below 8°C. Doffein[96] in the prawn, *Leander xiphias*, has shown that the complete darkness at 5–8°C for two or three days is responsible for the concentration of the pigments. Ih *Idotea*, Menke[97] found that the concentration of the chromatophores takes place at 12°C. Later, Smith[98] in the shrimp, *Macrobrachium aconthaxus*, reported that dispersion of pigment takes place at high temperature and concentration at low temperature. Fingerman and Tinkle[99], in *Palaemonetes pugio* and *P. paladosus*, have demonstrated that the white pigment which was more dispersed on a white background than on a black background, becomes concentrated on both the backgrounds with the increase in the temperature. Nagabhushanam[78,90] found that the red and black chromatophores of the shrimp, *Alpheus malabaricus*, concentrated with the rise in temperature. Further, Nagabhushanam and Rangarao[91] have reported that the black chormatophores of the prawn, *M. monoceros*, concentrated at high temperature.

In *C. weberi*[18], the red chromatophores concentrated on the white and black backgrounds with increase in temperature. On a black background, the red pigment remained fully dispersed from 15°C to 25°C; and above 25°C it began to concentrate. The concentrating activity was more at higher temperature (34°C). On a white background, the red pigment remained slightly dispersed at low temperature (15°C); and beyond this it was fully concentrated. A similar trend was observed for red chromatophores of *Alpheus malabaricus*[90], and black chromatophores of *Monoceros*[91]. However, the behaviour of the white chromatophores of *Caridina* was different. At all temperatures, the white pigment was more dispersed on a white background than on a black background. Thus it can be concluded, that at high temperatures, the red pigment is concentrated and the white pigment dispersed, while at the other end of temperature range, the pigments behaved in opposite manner. The yellow pigment of *M. kistnensis* concentrated on a white background with increase in the temperature and on a black background it dispersed at higher and low temperatures but showed an intermediate condition at room temperature[19].

Brown and Sandeen[48] in the fiddler crab, *Uca pugilatr*, have reported that the black pigment concentrated as the temperature increased above or decreased below 15°C, whereas, white pigment dispersed as the temperature increased or decreased below 20°C. Fingerman[49] has shown that the black pigment concentrated and red pigment dispersed with the increase in temperature in the crab, *Callinectes sapidus*. Further, Fingerman *et al.*[100] have demonstrated that the black pigment is concentrated at high temperature and dispersed at low temperature. Powell[101] in the crab, *Carcinus maenas*, has reported, that at high temperature, black pigment concentrated and white pigment dispersed, whereas, at low temperature both pigments dispersed. Nagabhushanam[86,78] and Nagabhushanam and Ranga Rao[102], concentrated and red pigment working on *Uca annulipes*, reported that black chromatophores concentrated and white dispersed with increase in temperature, while red chromatophores were slightly dispersed. Ranga Rao[103] in the crab, *Ocypoda macrocera*, has shown that the dark pigment dispersed at low and concentrated at high temperatures. This clearly reveals that these responses are of themoregulatory value. Diwan[83] in the freshwater crab, *Barytelphusa cunicularis*, has reported that with the increase in the temperature black chromatophores were concentrated and red dispersed. These findings do not agree with the earlier observations of Nagabhushanam[78,90] on *U. annulipes* and *A. malabaricus* where red pigment concentrated with increase in temperature, which are difficult to explain.

Zoaea

Very little is known about the chromatophore activity in crustacean larvae. Keeble and Gamble[45] first studied the chromatic activities in the larvae of *Hippolyte* and *Palaemon*, and showed that their zoaea exhibit a very uncertain background response. Carstam[65] observed a similar response of chromatopores in the eggs and zoaea of Norway lobster, *Nephrops morvegicus*. However, Pautsch[104] in the larvae of shrimp, *Crangon*, found a primary response of the chromatophores. Nagabhushanam[105–107] studied the chromatophore system in the larvae of *Diogenes bicristimanus*, *Sesarma reticulata* and *Upogebia affinis*, while Nagabhushanam and Sarojini[108] in developing crabs, *Gelasimus annulipes*.

Vasantha[18] has shown that the chromatophore system of the zoaea in *C. weberi* was poorly developed than that of the adults. Usually monochromatic chromatophores, which are regular in distribution, were observed in the larvae of *C. weberi*[18], *U. affinis*, *S. reticulata*[105,106] and *G. annulipes*[108]. The larval chromatophores of *M. kistnensis* are quite different from the adults. Majority of the chromatophores are dichromatic containing red and yellow pigments. Monochromatic white chromatophores were also observed.[19] The adult hermit crab, *Diogenes bicristimanus*, is devoid of any functional chromatophores. However, zoaea of the same possess distinct red chromatophores arranged on the body surface[109].

The larval chromatophores exhibit a diurnal rhythm for pigment migration. Nagabhushanam[106] and Nagabhushanam and Sarojini[109] observed that the black pigment of the zoaea of *S. reticulata* and the red pigment of *D. bicristimanus* dispersed during the day and concentrated by night.

Upon subjecting the zoea of C. *weberi*[18] to different intensity of illumination (0.10, 100 and 1760 ft.c.), the red pigment concentrated with the increase in illumination. However, the red chromatophores of the zoea of *D. bicristimanus*[108] and *U. affinis*[105] and black chromatophores of *S. reticulata*[106] disperse with the increase in the intensity of illumination.

Like the report of Keeble and Gamble[45], an uncertain background response has been observed in the zoaea of Norway lobster, *Nephrops*[65]; the shrimp, *Upogebia*[105]; the crab, *Sesarma*[106], and the hermit crab, *D. diogenes*[109]. However, in *Caridina*[18] and *Macrobrachium*[19] the red chromatophores showed a definite background adaptation. The chromatophores in *Caridina* reached maximum concentration on a white background and attained an intermediate state of dispersion on a black background within 15 minutes.

Moulting

Scheer and Scheer[110] observed cyclic changes in the degree of pigment dispersion in the chromatophores of the prawn, *Leander serratus*. Chassard[111] has demonstrated maximum concentration of the red and yellow pigments of *L. squilla* on white background only at the end of the moult cycle. Aoto[112] observed that the large red chromatophores of *Palaemon paucidens* are more or less dispersed, shortly before and during the moult cycle. Further, a large amount of red pigment dispersing and concentrating hormone is present in the eyestalks and central nervous organs at premoult than in intermoult, has been shown.

The red and white chromatophores of *C. weberi*[113] and yellow chromatophores of *M. kistnensis*[19] showed cycle changes during various stages of intermoult cycle. These chromatophores were more dispersed in the later stages of moult cycle (stage-D) than in stage B. The red and white pigments were somewhat more widely dispersed in animals in the later stage of the moult cycle (Stage-D) than in the premoult stage (Stage-B). However, in *M. kistnensis*[19] the yellow chromatophores were fully dispersed immediately after moult in stage A. A slight concentration was noted in stages B and C, and thereafter, the chromatophores were almost in a steady state upto the late proceedysial stage.

Nagabhushanam and Vasantha[113], while observing response for the chromatophores to longterm background adaptations, noticed that the same experimental animals responded to a changed background more slowly than others. The slow response was specially evident in the prawn with a soft carapace (soon after the moult) and in the ones with an opaque carapace (in the premoult stage). They have given two possible explanations: (1) more chromatophorotropins are being released from the eyestalk and central nervous organs during the moulting period, and (2) the chromatophores become more sensitive to chromatophorotropins.

Nagabhushanam and Vasantha[113] injected eyestalk extract and extracts of supraoesophageal ganglion with circumoesophageal connectives from premoult and postmoult to one eyed prawn, *C. weberi*. They found large amount of red pigment concentrating hormone in the eyestalk and supraoesophageal ganglia of the premoult animals than in the postmoult animals. Based on the above experiments, Nagabhushanam and Vasantha [113] put forth two hypotheses concerning the factors that determine the colour patterns. The first hypothesis is that the red pigment dispersing hormone is mainly present in the supraoesophageal ganglion, keeps the red chromatophores more expanded and is less responsible to a chaged background during the period shortly before moult. Secondly, the darker colour of the premoult prawns is due to the red pigment concentrating hormone, which is being stored in the eyestalk and supraoesophageal ganglion, but not released and thus do not affect ihe target organs, the chromatophores.

Endocrine Control of Chromatophores

Pouchet[42-44] found that the prawn was unable to change colour in response to different backgrounds when eyestalks were removed. In spite of negative results, other investigators also tried to explain nervous control of chromatophores[45,97]. The first evidence for endocrine control of chromatophores in crusteaceans came from Koller[2,3] and Perkins[4]. Koller[2,3] reported endocrine control of crustacean chromatophores based on blood transfusion experiments in the shrimp, *Crangon*, while Perkins[4] demonstrated that eyestalk removal caused a permanent dispersion of the red pigment of the prawn, *Palaemonetes*, and eyestalk extract administration caused rapid concentration. He also reported that the eyestalk is a soure of a hormonal substance which concentrates the red pigment of the prawn, *Palaemonetes*. Brown[114] gave the first evidence that chromatophorotropins, which exist in the central nervous organs outside the eyestalks of *Palaemonetes*, would induce blanching of dark, eyestalkless specimens. Furthermore, Brown *et al.*[61], reported a pigment dispersing principle in addition to red pigment concentrating principle which had been reported earlier[114]. They found that the duration of response to concentrating principle is shorter than the response to the dispersing substances.

In the prawn, *Leander serratus*, Panouse[115] reported that the sinus gland and central nervous organ extracts bring about red pigment concentrations. Scheer and Scheer[110,116] demonstrated that the red and blue pigment of *L. serratus* showed variations in the degree of pigment dispersion during the intermoult cycle. The eyestalks and central nervous organs control the concentrations of these pigments. Further, they have shown that eyestalk removal resulted in increased dispersion of each pigment but does not affect the cyclic changes and suggested that these changes must have been due to release of chromatophorotropins from post-commissure organs. Aoto[112], while working on large red chromatophores of *Palaemon paucidens*, found that substances that disperse and concentrate the pigment are present in the eyestalk, supraoesophageal ganglion and circumoesophageal connectives in large amounts in procedysis animals than in postecdys is animals. Pasteur[117] demonstrated that the removal of the sensory pore X-organ from both the eyestalks of *L. serratus* has a similar effect as that of sinus gland ablation. Red pigment remains fully expanded suggesting that the chromatophorotropins are axonally transported.

Among brachyurans, chromatic physiology of the fiddler crab, *Uca*, has been studied extensively. Carlson[56] reported that the removal of the eyestalks of *U. pugnax* and *U. pugilator* resulted in the concentration of the red pigment and migration of the red pigment takes place after eyestalk extract administration. Sandeen[118] found that the central nervous system of *U. pugilator* contains two chromatophorotropins, namely, a black dispersing substance and a white concentrating substance, both antagonistic to each other. Brown[119] found that the sinus glands and central nervous organs contain red pigment dispersing principle. Brown and Fingerman[120] showed that the red pigment dispersing substance differs from the white pigment dispersing hormone. Stephens *et al* [121] found the presence of three distinct areas on filter paper strips after electrophoresis of the sinus glands of melanin dispersing material and one dubious region of melanin concentrating hormone.

In three crabs, *Eriochir japonicus*[122], *Callinectes sapidus*[49],[123] and *Carcinus maenas*[123], removal of the eyestalks resulted in the concentration of the black pigment and maximum dispersion of the red pigment, thereby indicating that eyestalk contains both factors, which disperse melanin and concentrate the red pigment. These findings are just opposite to the observations of Brown[119] on *Uca* where the red pigment concentrated after eyestalk removal. An unusual case has been reported by Burgers[124], who found a crustacean chromatophore that does not appear to be controlled by even a single hormone. He observed that the melanophoric pigment in the crab, *Macropipus vernalis*, is under endocrine control but the red pigment appears to be an independent effect or responding directly to illumination.

Fingerman and Fitzpatrick[125] reported that the melanophores of the female *Uca pugilator* were more concentrated than those in the male. Further, the removal of the large cheliped from males caused them to become as dark as the females, but both became progressively darker if their walking legs were removed.

It is fairly known that all the decapod crustaceans exhibit either blanching or darkening of the body when both the eyestalks are removed. In the shrimp, *Crangon*, the body blanches and the tail darkens, after removal of both the eyestalks; and after 30 to 60 minutes, the body exhibits an intermediate colouration and the tail lightens[126]. Sinus glands of *Crangon* contain a body and tail blanching factor, where body blanching principle is present in the eyestalks of other crustaceans. The tail lightening principle was found to be present in the sinus glands of the prawn, *Palaemonetes*, however, absent in the sinus glands of the crabs, *Uca*, *carcinus*, *Pagurus* and *Libina*[14]. Brown and Scudamore[133] showed that all the sinus glands contain atleast two principles : (1) one blanches eyestalkless *Palaemonetes*, and (2) the other darkens *Uca*. According to Fingerman[37], sinus glands of *Uca* have atleast two principles and that sinus glands of *Crangon* and *Palaemonetes* possess a principle absent from sinus glands of crabs. He has given two possibilities : (1) sinus glands of *Crangon* and *Palaemonetes* possess three principles and sinus glands of crabs possess only two ; or (2) all sinus glands possess only two principles with one of the two differing in sinus glands of crabs from those of *Crangon* and *Palaemonetes*.

Noel[20] demonstrated a neural control of chromatophores in the prawn, *Processa edulis*. He found that after removal of one eyestalk of the specimen, blanching of half of the body from head to tail takes place. These observations are an exception to the general role that blanching and darkening in the individuals are hormonally controlled.

In the present review, endocrine regulation of chromatophores in the freshwater prawn, *Caridina weberi*, *Macrobrachium kistnensis*, freshwater crab, *Barytelphusa cunicularis* and a marine crab, *Gelasinus annulipes* have been discussed.

In *Caridina weberi,* all parts of the central nervous organs and eyestalks contain concentrating and dispersing hormone for the red pigment. Maximum red pigment dispersion occurred within 15 to 30 minutes with the abdominal nerve cord while in the case of the other tissues, maximum effect was observed only after 45 to 60 minutes. The circumoesophageal connectives contained the least amount of red pigment dispersing hormone. However, maximum concentration occurred within 15 to 30 minutes after injection of all the extracts. The white pigment dispersing hormone was found in all parts of the central nervous organs and eyestalks while white pigment concentrating hormone was found in two extracts of circumoesophageal connectives (Table-1).

Vasantha[18] found that the eyestalks and circumoesophageal connectives contain predominantly red pigment concentrating substances in approximately equal amounts. The abdominal nerve cord contains more red pigment dispersing principle, whereas, other portions of the central nervous organs possess less amounts of both red pigment concentrating and dispersing principles. These findings are in agreement with the earlier findings of Brown *et al.*[61] in *Palaemonetes.*

The abdominal nerve cord extracts have brought about dispersion of red pigment which remained for a short duration. It seems, that the abdominal nerve cord, though contains a small amount of dispersing principle, is able to disperse the pigment because of the absence of concentrating principle. The ratio of red pigment dispersing to red pigment concentrating substance is higher enough in the abdominal nerve cord to bring about dispersion. Vasantha[17] found that with the increase in the concentration of eyestalk extract, the dispersion of red and white pigment and the concentration of the red pigment increases. Similar trend was observed with the thoracic ganglion extract, but there was no change in the red pigment concentrating activity. However, the red pigment concentrating hormones showed higher values than the red pigment dispersing values in the thoracic ganglion and abdominal nerve cord.

In *Macrobrachium kistnensis*, Nagabhushanam and Keshavan[126] have reported that both eyestalks and central nervous organs contain chromatophorotropins. The yellow pigment dispersing hormone was found in the eyestalk and other parts of central nervous organs. However, yellow pigment concentrating hormone was not recorded in the circumoesopageal connections and abdominal ganglia (Table-2). The eyestalk contains both dispersing and concentrating factors, though the amount of dispersing hormone is less.

The eyestalk contains more yellow pigment dispersing hormone than that in other central nervous organs. The supraoesophageal ganglion contains less amount of yellow pigment dispersing hormone when compared to thoracic

Table-1 Activity values of chromatophorotropins in the eyestalk and central nervous organs of the freshwater prawn, *Caridina weberi* (after Vasantha, 1968).

Tissues	RPDH	RPCH	WPDH	WPCH
Eyestalk	1.3 ± 0.27	4.0 ± 0.54	20.4 ± 1.42	0
Supraoesophageal ganglion	2.8 ± 0.35	3.7 ± 0.32	16.5 ± 0.95	0.9 ± 0.06
Circumoesophageal coonnectives	0.3 ± 0.14	4.4 ± 0.42	9.6 ± 0.47	1.8 ± 0.44
Thoracic ganglion	5.5 ± 0.37	1.2 ± 0.31	20.4 ± 1.52	0
Abdominal ganglion	3.8 ± 3.42	0.4 ± 0.02	23.6 ± 1.85	0

RPDH= red pigment dispersing; RPCH= red pigmeut concentrating; WPDH= white pigment dispersing; and WPCH= White pigment concentrating hormone.

Table-2 Activity values of chromatophorotropins in the eyestalk and central nervous organs of the freshwater prawn, *Macrabrachium kistnensis* (after Nagabhushanam and Keshavan, 1974).

Tissues	YPDH	YPCH
Eyestalk	5.3	1.0
Supraoesophageal ganglion	1.4	0.6
Circumoesophageal connectives	2.2	–
Thoracic ganglion	3.5	0.4
Abdominal ganglion	3.9	–

YPDH= Yellow pigment dispersing; and YPCH= Yellow pigment concentrating hormone.

Table-3 Activity values of chromatophorotropins in the eyestalk and central nervous organs of the freshwater crab, *Barytelphusa cunicularis* (after Diwan and Nagabhushanam, 1974).

Tissues	BPDH	RPDH	RPCH
Eyestalk	12.50 ± 2.45	6.00 ± 1.65	1.80 ± 0.45
Supraoesophageal ganglion	8.50 ± 1.90	4.30 ± 0.84	3.20 ± 0.57
Circumoesoph ageal connectives	2.35 ± 0.84	1.90 ± 0.55	4.20 ± 0.68
Thoracic ganglion	6.30 ± 0.65	3.00 ± 0.80	6.50 ± 1.45

BPDH= black pigment dispersing; RPDH= red pigment dispersing and RPCH= red pigment concentrating hormones.

or abdominal ganglia (Table-2). The yellow pigment concentrating hormone was found in the eyestalk, supraoesophageal ganglion and thoracic ganglion. Probably it was not present in an active form in the circumoesophageal connectives and abdominal ganglia or the quantity of the hormone was so small that it could not be detected easily. The yellow pigment dispersing activity increased progressively with the increase in the concentration of eyestalk and supraoesophageal ganglia extracts. The rest of the central nervous organs showed that the dispersing activity decreases progressively with increase in the concentration (Table-2).

Eyestalks and all parts of the central nervous organs contain black pigment dispersing and red pigment concentrating hormone in the freshwater crab, *Barytelphusa cunicularis*[74]; and black pigment dispersing hormone was more in the eyestalks and supraoesophageal ganglion. The red pigment dispersing hormone was found to be maximum in the eyestalks only. However, black pigment concentrating hormone was not recorded either from the eyestalkless animals or from intact specimens adapted to black background. It contains dispersing factors for both red and black chromatophores and concentrating factor only for the red chromatophores in all parts of the central nervous orgons (Table-3).

In *Uca pugilator*[118,120], black pigment permanenty concentrated following the eyestalk ablation and dispersed after eyestalk extract administration. However, Enami[128,129] demonstrated that dispersion of black pigment in the crab, *Sessarma hoematocheir*, takes place after eyestalk ablation. In *B. cunicularis* also, dispersion of black pigment takes place after eyestalk ablation. These observations are just opposite to the findings of other investigations on *Uca*[118,120].

Findings of Diwan and Nagabhushanam[74] on the presence of and distribution of red pigment dispersing and concentrating hormones in *B. Cunicularis* are in agreement with the observations of Brown[119], Matsumoto[122], Fingerman[49], Powell[123] and Nagabhushanam[86] on other decapod crustaceans.

Nagabhushanam[86] reported maximum concentration of black pigment takes place after eyestalk ablation in *G. annulipes*. Nagabhushnam[107] further described that the high concentration of black pigment dispersing hormone is present in the optic ganglia, sinus gland and supraoesophagal ganglion. The thoracic ganglion circumoesophageal connectives contain a low concentration of this hormone.

Removal of the eyestalks resulted in red pigment concentration in *G. annulipes*[78]. Later, Nagabhushanam[107] demonstrated that the red pigment is controlled by two factors, one concentrates and the other disperses. He found the highest activity of the red pigment concentrating factor in the thoracic ganglia extract, and the highest red pigment dispersing activity in the optic ganglia. The observations of Nagabhushanam[86,78,107] on the distribution of red pigment dispersing and concentrating hormones of *G. annulipes* are similar to the findings of Brown[119] on *U. pugilator*. Nagabhushanam[107] injected various central nervous organ extracts into black adapted forms and eyestalkless *G. annulipes.* He observed white pigment dispersing activity in the total eyestalk, optic ganglia, sinus gland and supraoesophageal ganglion. However, circumoesophageal connectives and thoracic ganglion did not show pigment dispersing activity. Considerable white pigment concentrating activity was obtained with extracts of total eyestalk, optic ganglia and circumoesophageal connectives. Very little activity was found with extracts of supraoesophageal ganglion and thoracic ganglion. However, white pigment concentrating activity was absent in the sinus gland extract.

Mode of Release of Chromatophorotropins

Abramowitz and Abramowitz[130] reported that chlorides of Li, Na, K and Mg caused rapid concentrations of the dispersed black chromatophores of *U. pugilator.* Fingerman *et al*[131]. observed the effect of osmotic pressure and actions of cations on the response of the melanophores in *U. pugnax,* to the melanin dispersing principles from the sinus gland. They have demonstrated that the activity of melanin dispersion was more in dilute sea water than in concentrated one. Further, they have shown that monovalent cations such as Na, K, Li were necessary for a maximal response of the melanin dispersing principle. However, Koller[132] and Knowles[14] have reported that calcium is a necessary cation to provoke pigment migration. Knowles[14] found that the chromatophores in the isolated carapace of *Palaemon* concentrated in darkness and failed to re-expand under illumination. However, a mixture of $CaCl_2$ and $NaCl_2$ concentrated the chromatophores in darkness and re-expanded in light. Nagabhushanam studied the effect of osmotic pressure and cations in *G. annulipes*[107].

Ions.

Nagabhushanam and Vasantha[134] and Nagabhushanam and Keshavan[127] treated isolated pieces of carapace of the prowns *C. weberi* and M. *kistnensis* respectively to 0.25, 0.5, 0.7 and 1% solutions of NaCl. In *C. weberi* an increase in the red pigment dispersing activity was noticed when isolated pieces of carapace were treated with eyestalk extract prepared in 0.25% NaCl. However, peak activity for the yellow pigment dispersion in *M. kistnensis* was observed with eyestalk extracts prepared in 0.75% NaCl. Novales[135] found that 50% Ringer's solution was more effective in producing melanin dispersion in melanophores. Fingerman *et al.*[131] obtained good dispersion of melanophores with 10 and 50% sea water. Results on *C. weberi* are similar to the findings of Novales[135] and Fingerman *et al.*[131] that a hypertonic solution had a better effect on dispersion of the chromatophores. However, unlike the above findings, the yellow chromatophores of *M. kistnensis* responded to higher NaCl concentration. Fingerman *et al.*[131] have explained pigment dispersing activity in terms of water entry into the chromatophores.

Novals[135] reported that Na alone was responsible for the response of melanophores to intermedin in the frog. However, Fingerman *et al.*[131] found that NaCl could be substantiated by other monovalent ions like K and Li for maximum responses to the melanin dispersing principles in *U. pugnax.* In *C. weberi* and *M. kistnensis* out of the Na, K, Ca, and Mg tested, inhibition of dispersion with Ca and Mg was observed. However. Fingerman and Connell[136] found that the red pigment dispersing hormone in *Palaemonetes* is Ca dependent. Further, they have suggested that the action of this hormone would be because of an increase in the rate of Ca influx; and increased concentration of Ca inside the erythrophores would then save to activate the pigment dispersing hormone. Fingerman and Connell[136] have reported that the red pigment concentrating hormone is Na dependent.

Fingerman and Connell[136] by using tetradoxin and ouabain, which are known to block Na entry into cells and Na transport out of the cells, respectively, have proposed that the red pigment concentrating hormone could act in two ways *i.e.* (i) it could stimulate the Na pump, associated with the cell membrane which is inhibited to ouabain thus increasing the amount of Na ion being pumped out of the erythrophore, while not interfering as with the influx of Na; and (2) block influx of Na which can also be blocked by tetrodoxin, while Na pump continuesous to pump out Na at a steady rate.

Adenosine Triphosphate.

Lerner and Takashi[137] reported that ATP brought about dispersion of melanin granules in the melanophores of the isolated frog skin indicating that energy is used during migration of pigment. However, inhibitory response of ATP was observed in the isolated pieces of carapace for the red chromatophores of *C. weberi*[134], yellow chromatophores of *M. kistnensis*[127], and of the lizard, *Anolis*[138]. The lightening effect in the lizard is due to the action of ATP through the Ca present in the Ringer's solution. It seems that the cell surface plays a role in the migration of the pigments within chromatophores.

Adenosine 3' 5' Monophosphate (cyclic).

Cyclic AMP, which has been shown to be an intracellular mediator for the action of several hormones in vertebrates, mimics the action of intermedin on the melanophores of amphibians [139,135,140]. These observations are similar to the findings of Fingerman[82] in *Palaemonetes.* This response is not Na dependent, and the hormone may cause an increase in the rate of influx of Ca ions into the chromatophores. The higher concentration of Ca ions within the chromatophore would activate adenyl cyclase which catalyses the synthesis of cyclic AMP from ATP. The amount of cyclic AMP synthesized would depend upon the amount of red pigment dispersing hormone and the amount of Ca entering the chromatophore. Cyclic AMP would then trigger the pigment dispersion in proportion to the amount that was synthesized and would in turn be degraded to 5' AMP. Thus AMP acts as a second messenger whereas the chromatophorotropin is the first.

Biogenic Amines

The studies of Aoto[21], Burgers[124] and Bauchau and Mengeot[23] indicated that in addition to the well established peptide neurosecretory products some inodolealkylamines may also be involved in the chromatophysiology of crustaceans. It has been observed that biogenic amines like serotonin, epinephrine and norepinephrine caused pigment dispersion in the red chromatophores of the prawn, *Palaemon squilla*[24]. The studies of Murdock,[25] (1) Gerschenfeld[26] and Kerkut[27] on the presence of neurotransmitters in the crustaceans has given some clue on the role of transmitters in the chromatic physiology of crustaceans. A survey of literature shows that three groups of neurotransmitters are present in crustaceans, (1) monoaminergic transmitters and (2) cholinergic transmitters and (3) acidicamines. The mono-aminergic system comprises the serotoninergic system and the adrenergic system.

The monoaminergic transmitters are formed during the metabolic degradation of tryphan; which successively gives rise to 5–HTP and then 5–HTM serotonin. Burgus[124] and Noel[20] demonstrated red pigment dispersion in *P. serratus* and *Processa edulis* respectively after administration of 5–Hydroxytryptophan (5–HTP).

Nagabhushanam[141],[142] found dispersion of red chromatophores after serotonin administration in the shrimp, (*A. malabaricus*), and crab, (*G. annulipes*). Further, Nagabhushanam[143] observed that administration of adrenaline in *G. annulipes* also brings about dispersion of red chromatophores. Based upon these observations he postulated that serotonin and adrenaline may have some role in the regulation of the pigment dispersion.

When 5–hydroxytryptamine (5–HT, serotonin) was injected into specimens, both eyestalkless and intact, of *Uca pugilator*, whose red chromatophoric pigment was maximally concentrated, dispersion of this pigment was produced[144],[145]. However, when 5–HT was injected into isolated legs having maximally concentrated red pigment it had no effect on the red chromatophores. In contrast, red pigment dispersing hormone from the eyestalks is effective both *in vivo* and *vitro*. The results of these experiments with 5–HT led Rao and Fingerman[144] to formulate the hypothesis that atleast one of the normal functions of 5–HT in this species of fiddler crab is to control the release of red pigment dispersing hormone (RPDH) from the neurosecretory cells, where this hormone is synthesized and stored. A similar mode of action is postulated in the case of *Cambarellus*[146]. The melanophorotropic affect of 5–HT in *Carcinus* also appeare to be mediated by the neuroendocrine system[147] but little has been done to assess whether this action involves the release of black pigment dispersing hormone.

Subsequent studies in this area have focused on evaluating the possible role of 5–HT as a neurotransmitter controlling the release of RPDH in *Uca pugilator.* The eyestalks and brain of *Uca* were shown to contain 5–HT; the 5–HT levels in these tissues varied in relation to background adaptation and in response to treatment with pharmacological agents[148]. The levels of RPDH and 5–HT[149] as well as the level of 5-hydroxytryptophan decarboxylase in the eyestalks of fiddler crabs[150] showed 24–hour variations in relation to the rhythmic colour changes involving the erythrophores. Additionally, the background adaptive erythrophore responses in the fiddler crab were altered by treatment with drugs that are known to affect 5–HT levels and/or 5–hydroxytryptaminergic systems[85,151,152].

Thus, it can be summarized that monoamine oxidase (MAO) inhibitors and 5–HT uptake inhibitors were able to potentiate erythrophore pigment dispersion in crabs transferred from a white background to a black background and also inhibit (or suppress) erythrophore pigment concentration in crabs transferred from a black background to a white back ground. Conversely, 5–HT deletors and 5–HT antagonists were able to inhibit erythrophore pigment dispersion and potentiate erythrophore pigment concentration. The above observations provide strong support to the views that 5–HT serves as a neurotransmitter in controlling the release of RPDH in *Uca.*

Dopamine

When injected into *Uca pugilator*, dopamine (DA) elicits erythrophore pigment concentration. This effect is evident *in vivo* but not when tested on isolated legs *in vitro*, indicating that DA has no direct effect on the erythrophores[68]. Further analysis showed that DA elicits erythrophore pigment concentration by stimulating the release of RPCH (red pigment concentrating hormones) from the neuroendocrine system. Because of this RPCH releasing effect, DA was able to antagonize the RPDH mediated action of 5–HT in *U. pugilator*[68].

Fingerman and Rao[145] noted that the RPDH mediated action of 5–HT in *Uca pugilator* can be antagonized by lysergic acid diethylamide (LSD) which produced erythrophore pigment concentration *in vivo* but not *in vitro*. Subsequent work showed that LSD, isolysergic acid amide, and psilocin[146] as well as DA and certain derivatives of lysergic acid-ergocornine, L-ergocryptine, and bromocryptine are able to trigger erythrophore pigment concentration when injected into *U. pugilator* but not when tested on isolated legs *i.e. in vitro*[153]. Thus, in fiddler crabs, LSD and certain other derivatives of lysergie acid seem to act as DA agonists and/or as 5–HT antagonists on the neuroendocrine systems. Since these compounds bring about erythrophore pigment concentration in a rapid manner (within 5 to 15 minutes after injection) their effect appears to be mediated largely by the release of RPCH (*i.e.* DA agonist action) rather than by a mere inhibition of RPDH release (*i.e.* 5–HT antagonist action).

That DA triggers the release of RPCH, is supported by more recent tests with DA agonists and antagonists[151]. Thus, background-adaptive erythrophore pigment concentration is potentiated by ADTN (2 amino-3,4 dihydroxy 1,2,3,4-tetrahydronophthalene hydrobromids), a well known DA agonist, and inhibited by spiroperidol (DA antagonist) and r-hydroxybutyrate (DA release inhibitor).

Norepinephrine

When injected into *Uca pugilator* norepinephrine (NE) triggers melanophore pigment dispersion. This effect is evident *in vivo* but not when tested to isolated legs, *i.e. vitro*, indicating that NE has no direct effect on the melanophores[154]. Further analysis[155,156,157] indicated that NE triggers melanophore pigment dispersion by stimulating the release of MDH (Melanophore dispersing hormones). The possibility that this hormone releasing action of NE involves α_1 adrenoceptor activation. For example, in crabs transferred from a white background to a black background, the normally occurring melanophore pigment dispersion is inhibited (or suppressed) by adrenoceptor blockers and NE uptake inhibitors; the pigment dispersing response of melanophores was unaffected by β-adrenoceptor blockers. On the basis of these and other results, and in light of the current knowledge of mechanisms involved in α_1 -adrenergic responses, a schematic diagram is prepared to indicate the possible noradrenergic regulation of MPH release (Fig.3).

Serotoninergic monoamines, serotonin and 5–hydroxytryptophan evoked red pigment dispersing response in the erythrophores of the prawn, *Caridina rajadhari*[29]. Tryamines, dopamine, and norepinephrine produced the red pigment concentrating response while epinephrine caused slight red pigment dispersion. Acetylcholine, the cholinergic neurotransmitter, in high doses evoked the red pigment concentration. *In vitro*, all these biogenic amines failed to evoke any pigment migrating response, indicating their indirect effect on the erythrophores. All these results lead to the conclusion that the erythrophoric pigment migration in the prawn, *C. rajadhari*, is regulated mainly by serotoninergic and adrenergic monoamines and partly by the cholinergie transmitters.

The alkaloids, reserpine, caffeine, and theophylline evoked the erythrophoric pigment dispersing response in *C. rajadhari*[29] *in vivo*, but did not have any action when studied *in vitro*. Injection of colchicine produced the erythrophoric pigment dispersing response. *In vitro*, it appeared to have inhibitory action on the hormonally induced red pigment dispersion and concentration. These various effects of alkaloids indicate that they have some indirect action on the erythrophores, presumably by triggering the release of chromatophorotropins. *In vitro*, the inhibitory action of colchicine may not be due to the disruption of microtubules but *via* some other mechanism, as it required very high concentrations to produce the particular response[29].

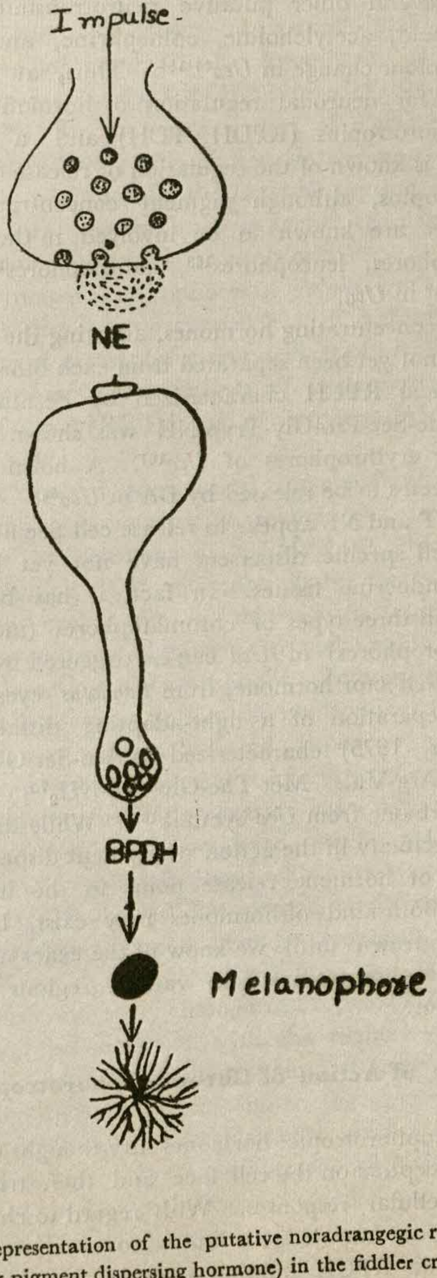

Fig. 3 Diagramic representation of the putative noradrangegic regulation of the release of BPDH (Black pigment dispersing hormone) in the fiddler crab, *Uca*. In this scheme, norepine phrine (NE) stimulztes BPDH release by acting 1-adrenoreceptors.

Unresolved problems.

Although NE, 5-HT and DA are able to trigger hormone mediated colour changes in *Uca*, several other putative neurotransmitters-glutamic acid, Y-amino butyric acid, acetylcholine, epinephrine, and octopamine-do not seem to elicit any colour change in *Uca*[68,154]. Thus, at this time, pharmacological evidence for neuronal regulation of hormone release is available only for erythrophorotropins (RPDH, PCH) and a melanophorotropin (MDH). Nothing is known of the regulation of release of leucophorotropins and xanthophorotropins, although pigment concentrating and pigment dispersing hormones are known to be involved in the control of different types of chromatophores, leucophores[158], xanthophores[159], melanophores[160] and erythrophores[119] in *Uca*.

The pigment concentrating hormones, affecting the different chromatophores of *Uca* have not yet been separated from each other[159], but a synthetic preparation of *Pandalus* RPCH characterized by Fernlund and Josefsson[67] as: Glu-Leu-Asn-Phe-Ser-Pro-Gly-Tryp-NH was shown to cause cell specific concentration in the erythrophores of *Uca*[161]. A hormone with a similar cellular specificity seems to be released by DA in *Uca*[68].

Although 5–HT and NE appear to release cell pecific RPDH and MDH respectively, such cell specific dispersers have not yet been isolated from crustacean neuroendocrine tissues. In fact, it has been reported that, pigment dispersion in three types of chromatophores (melanophores, leucophores, and erythorophores) of *Uca* can be triggered by any one of several forms of pigmentary-effector hormone, from *Pandalus* eyestalks[162,163], including a synthetic preparation of a light-adapting distal retinal pigment hormone (Kleinholz, 1975) characterized as Asn-Ser-Gly-Met-Ple-Asn Ser-Ile-Len-Gly-Ile-Pro-Arg-Vul. Met-The-Glu-Ala-NH$_2$[70], as well as by a partially purified hormone from *Uca* eyestalk[164]. While these studies indicate a lack of cellular specificity in the action of pigment disperses, pharmacological investigations of hormone release point to the involvement of cell specific dispersers. Both kinds of hormones may exist, but definitive conclusions cannot be drawn until we know of the exact number and cellular specificity of chromatophorotropins from various regions of the crustacean neuroendocrine system.

Mode of Action of Chromatophorotropins

Crustacean chromatophorotropic hormones are thought to exert their effect by first binding to receptors on the cell face and then triggering a series of events leading to cellular responses. With regard to chromatophootropin-receptor interactions, our knowledge is limited to a report on the extractability of a heat-labile RPCH binding material from the epidermal tissues of *Palaemon*[165]. Additional work in this area is expected from the recently prepared[126] I-labelled RPCH analog[166] with respect to intracellular changes associated with hormones triggered pigment translocations ; and several different hypotheses have been proposed over the years.

Hormone induced pigment concentration in the erythrophores of *Palaemonetes vulgaris* was found to be inhibited by ouabain, enhanced by tetrodotoxin[136] and accompanied by hyperpolarization[167]. These observations led Fingerman[168] to propose that RPCH acts by stimulating the Na/K pump in erythrophore membrane and causing hyperpolarization. Recent studies, however, did not provide support for this hypothesis. For example, in the erythrophores of *Ocypode pallidula*, RPCH action did not involve changes in membrane potential[169]. In the erythrophres of *Palaemonetes pugio*, RPCH action did not appear to involve stimulation Na/K pump but seemed to be exerted via promotion of Ca influx, the Ca transporting ionophore A 23187 mimicked RPCH action, and both these agents required extracellular Ca far causing erythrophore pigment concentration[34,170].

When A 23187 treated erythrophores of *Palaemonetes* were placed in Ca free saline, pigment dispersion occured[34,170]. This finding, alongwith the previously demonstrated pigment dispersing effect of cAMP (cyclic adenosine, 3, 5, monophosphate) on erythrophores of *Palaemonetes*, contributed to the current belief that RPDH action involves cAMP production and Ca efflux [34,170].

Earlier work on hormone induced pigment dispersion in brachyuran melanophores pointed to a nonspecific monovalent cation requirement[131] and to a possible involvement of CAMP[28,32], showing that cAMP, cGMP (cyclic guanosine 3',5' monophosphate) and the calcium ionophore A23187 are each able to minic a partially purified eyestalk hormone in eliciting pigment dispersion in melanophores, leucophores and erythrophores in isolated legs of *Uca Pugilator*. Interestingly, when perfused alongwith the eyestalk hormone both cyclic nucleotides and A23187 seemed generally to potentiate hormone induced dispersion in the melanophores and leucophores, but inhibit dispersion in erythrophores ; the degree and duration of potentiation or inhibition exerted on a particular chromatophore type varied with the dosage of the tested agents. Methylxanthines (e.g. caffine, theophylline and aminophylline), which are known to affect cellular cAMP and Ca levels, also showed dose related variations in the potentiation or inhibition of hormone-triggered pigment movements within crustacean chromatophores. These may be modulated/mediated by alteration in cyclic nucleotide metabolism and/or by changes in Ca distribution or flux (Fig. 4). This mode of action is consistent with the demonstrated roles of cyclic nucleotides and Ca in modulating the responses of various effectors.

Ultrastructural studies[173] have shown the presence of cytoskeletal structures such as microtubules, microfilaments, and/or irregular filamentous elements[34] in crustacean chromatophores. Colchicine (a well known microtubule poison) and cytochalasin B (which can disrupt microfilaments) are shown to inhibit pigment movements in crustacean chromatophores[31,32,34,178,174,164].

FIG-4

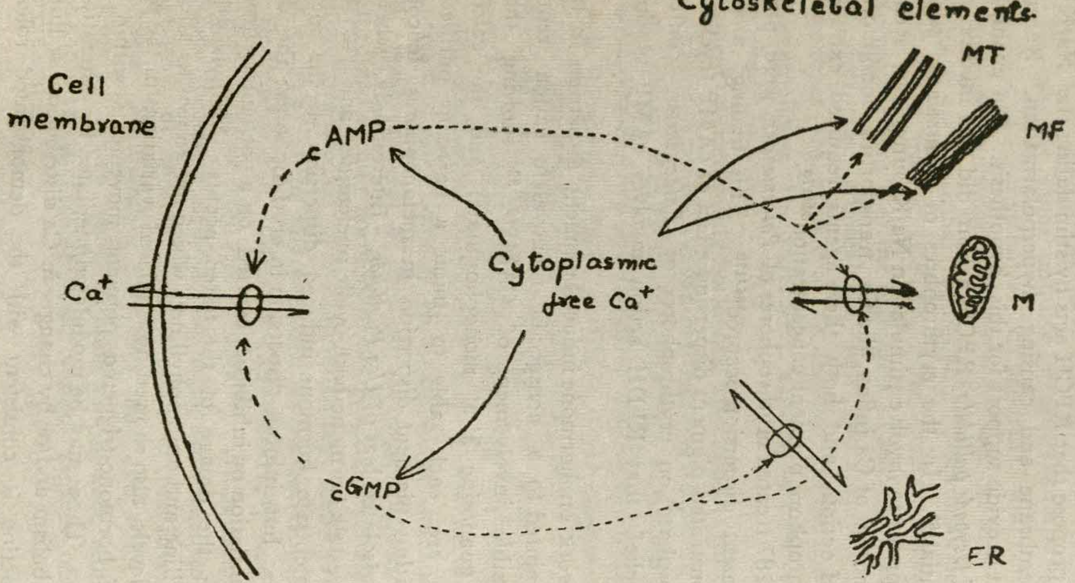

Fig. 4: A schematic representation of the possible roles of Ca+, cAMP and cGMP in modulating the intracellular activity in chromatophores. Hormone induced pigment movements seem to be modulated/mediated by alterations in the levels of cyclic nucleotides and ca. These agents may have mutually modulatory effects on each other and also help regulate the intengrity/function of cytoskeletal elements such as microtubules (MT) and microfilaments (MF). The endoplasmic reticulum (ER) and mitochondria (M) could serve as storoge/release sites for intracellular Ca.

That these observed inhibitory effects area result of disruption of microtubles and/or microfilaments, has not been demonstrated convincingly in any of the cases. For example, although cytochalasin B inhibited pigment concentration in the ovarian chromatophores of *Palaemonetes*, ultrastructure study of the treated cells did not show disruption of microfilaments[175]. The inhibitory effect of colchicine on pigment movements in erythrophores of *Palaemonetes* were evident only when this drug was applied at concentrations much higher than those required for microtubular disruption[148],[175]. Additionally, in tests with melanophores of *Uca*, lumicolchicine which does not interact with microtubules) proved to be as effective as colchicine in inhibiting pigment movements[176]. These findings, along with the possibility that colchicine and cytochalasin B may act at sites other than cytoskeletal elements (as discussed by Lambert and Fingerman)[34], point to a need for additional work in this area.

In view of the unclear roles of microtubules and microfilaments, as well as due to the observed association (connections) between endoplasmic reticular elements and pigment granules, it is thought that the networks of endoplasmic reticulum may aid in a pigment movements[177]. The current evidence is inadequate to suggest a force-generating role for the endoplasmic reticulum, but the possibility that it aids in the pigment translocating processes by serving as a storage/release site for Ca mertis consideration.

Perspectives

The release of chromatophorotropins from the crustacean neurosecretory system is thought to be controlled by neural signal. Recent pharmacological studies on fiddler crabs indicate that biogenic amines, such as 5-HT, DA, and NE, play a role in triggering the release of some of the chromatophorotropins. Future work in this area should focus on the cytochemical localization of the putative 5-hydroxytryptaminergic, dopaminergic, and noradrenergic inputs to the neurosecretory system, as well as identification of exact cellular sources of chromatophorotropins, and elucidation of the total number and cellular specificity of chromatophorotropins from various regions of the neuroendocrine system.

With regard to the mode of action of chromatophorotropins, current evidence points to the importance of cyclic nucleotides and Ca in modulating the intracellular events leading to pigment movements. But the precise roles of cyclic nucleotides and Ca, as well as details of the pigment-translocating mechanism remain to be elucidated.

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6

PLANT NEMATODES

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Introduction

Plant nematodes continue to compete with humans for almost all types of vegetation and crops. Our knowledge about them has grown at a much faster rate during the recent years with man's ever increasing concern for food crops. The account presented here is an attempt to bring to surface the current position regarding some important aspects of plant parasitic nematodes and different approaches towards their control.

Plant parasitic nematodes, frequently even the species without any close relationship, are found almost on all types of vegetation and soil. Some species like *Heterodera schachtii* are restricted to a few species of plants, but others like *Meloidogyne radicola* and *M. marioni* are known to infest over 1,000 varieties of plants. The damage by plant nematodes is not confined to the root system alone, it also extends to other organs of the plants, such as tubers, corms, rhizomes, stems, leaves, buds, flowers and fruits. Losses in crop yields are quite often attributed to low soil fertility, when these are actually due to nematode attack exhibiting no discernible symptoms of damage. The magnitude of the damage, all over the world, is difficult to assess. However, with the use of modern nematicides the impressive rise in crop yields in U.K.,U.S.A., Europe and Russia, the destructive power of plant nematodes is becoming more and more evident.

The nematologists in Netherland, U.S.A., Austria, U.K., Germany and Russia had realised the destructiveness of nematodes much earlier than those of the many other developed and under-developed countries. Lack of this awareness in other countries towards the destructive role of plant nematodes in crop economy is subscribed to the insidious nature of damage by nematodes to plants in contrast to the spectacular damage with evident symptoms, done by viral, bacterial and fungal diseases. The study of plant nematodes gained maximum impetus during the preceding four decades with the discovery of nematicidal properties of the mixtures of dichloropropenes and dichloropropanes by Carter(1943)and followed by a low cost nematicide, ethylene bromide, by Christie (1945). Currently, besides U.S.A., U.K., Russia and many other European countries, active work is in progress in Japan, Canada, Israel, South Africa, Newzealand and Australia. In India, though a beginning of study on plant parasitic nematodes was made at Aligarh Muslim University in 1959, yet the foundations of organised research on plant nematology were laid in early sixties much due to the efforts of Prof. F.G.W. Jones and late Dr. J.B. Goodey from Rothamsted Experimental station U.K., and Dr.D. Raski from the Univeristy of California U.S.A.

Historical Appraisal

Needham (1743), in a letter to the president of the Royal Society, London, is credited with the first account of a plant nematode forming galls in earcockles of wheat. Roffredi (1775) demonstrated the relationship between the nematode and gall formation in wheat, and Steinbuch (1799) named this nematode as *Vibrio tritici*. It is now known as *Anguina tritici* (Steinbuch, 1799) Chitwood, 1935. This discovery stimulated the interest in plant nematodes among many workers, and led to the description of numerous species. The account of these species is well documented in a series of classical monographs by Dujardin (1845), Bastian (1865), Schneider (1866) Butschli (1873), de Man (1884), Ritzema-Bos (1888) and Cobb (1893).

Plant nematology developed at a very rapid pace during the first half of the present century. With the contributions of J.G. de Man and J. Ritzema Bos in Netherlands; N.A. Cobb and G. Steiner in U.S.A., H. Micoletzky in Austria, T. Goodey in U.K.,H. Goffart and E. Reinmuth in Germany, I.N. Filipjev in Russia and many other workers in different countries, plant nematology covered vast grounds and acquired new dimensions. Goodey (1933) is the first to put the subject together in his memorable publication 'Plant parasitic nematodes and the diseases they cause'. In this publication, the plant parasitic nematodes are placed under 4 genera though 6 other are listed. Since then, the number of genera, embracing 2260 species, has swelled to 207 (Bowen, 1977). The plant nematode fauna reported from India is represented by 26 genera covering

156 species (Anon, 1970).

We never had such a clear understanding of problems associated with diversified fields of plant nematology as we have today. Our knowledge of plant nematology is enriching every day as evidenced by more than 1,200 research articles published every year. Many a treatises, bulletins, monographs and books (Chitwood and Chitwood, 1950; Southey, 1959, 1970 Christie, 1959; Sasser and Jenkins, 1960; Tarjan, 1960; Kiryanova, 1961; Baker, 1962; Parmanov, 1962; Jenkins, Mai and Stessel, 1963; Wallace, 1963, 1973; Zuckerman, Mai and Rhode, 1971; Webster, 1972; Kiryanova and Kraal, 1980; Decker, 1981; Anon, 1984 and many others) have proved indispensable tools for training and research in plant nematology.

Taxonomy and Morphology

Possibly, one of the factors contributing to nematode classification being by no means stable could be attributed to nematodes being studied independently by those interested in (i) free living forms, (ii) parasitic nematodes of vertebrates, (iii) parsitic nematodes of invertebrates and (iv) parasitic nematodes of plants. It is to the credit of Chitwood and Chitwood (1950) to put the marine, soil and parasitic nematodes into a single system to lay foundations of modern nematode classification. This was followed, in fifties, by a set of monographs in succession by Russian workers. The translation of some of these paved way for better understanding and attempting classification on a more rational basis.

The nematodes are now divided into two groups, i.e. class Secernentea, mainly comprising of soil and parasitic forms; and class Adenophorea consisting mainly of & marine forms. The affinities and evolutionary relationship between these two classes are still a matter of judgement and convenience. In the absence of palaeontological evidence, the arrangement of nematode species into systems of classification is far from exact, and is primarily based on reasoning, experimentation and convenience. Efforts to derive phylogenies of better understood groups of plant nematodes, based on their trophic specialisation and co-evolution of their host, have recently been attempted (Krall and Krall, 1978; Stone, 1980). It is hoped that more and more developmental studies, co-relation between different juvenile and adult stages will contribute towards determining the phylogenies on a more authentic basis.

The past fifty years are full of discovery and description of numerous species of nematodes. Many new species are being described each year and will continue to be described, since not all the plants have been surveyed for nematodes attacking them. Roughly, 2,500 species have been described, and many thousand still remain to be described. Therefore, it is quite likely that nematode classification will continue to remain in a fluid state for many years to come. The need for more and more work on the taxonomy of plant nematodes has been stressed (Anon, 1979b). However, there has been enormous increase in our knowledge of nematode systematics and relationship.

Identification of plant nematodes at family and generic level normally involves no such problem as at species level; where the minute size of specimens, absence of skeletal structures and difficulties in processing the specimens, if not obscure, render the identification extremely difficult. With advances in optics and techniques, such as transmissson and electron microscopy, many structural patterns that could not be determined with certainity under light microscopy are now determinable with accuracy. The unravelling of a wide range of structural details, by newer innovations, will result in formation of clear concepts on relationship, evolution and systematics. In oddition to gross compartive morphology and biology, information from varied areas like nematode physiology, biochemistry, parasitology, etc. will continue to contribute towards a better understanding and working of nematode phylogeny.

Host-parasite Relationship

Dropkins (1955) classified the response of the host-tissues into following categories: (i) Cell hypertrophy, (ii) hyperplasia, (iii) cell necrosis, (iv) dissolution of parts of cell wall and (v) alteration in tissue differentiation. Ectoparasites feed for a short duration, and inflict injury at the feeding site. The sedentary females feed for a month or more. During feeding, they extrude saliva into the host tissues which is compatible with the contents of the host cell. The saliva contains hydrolytic enzymes such as amylase, invertase, protease and pectinase. These enzymes help the plant nematodes in penetrating the host-tissues and partially hydrolysing the cell contents to render them assimilable. Cellulolytic and pectinolytic enzymes have also been discovered in the salivary secretion of many species of plant nematodes. Probably, these enzymes help in penetrating the cell walls of the plants. Krusberd (1963) maintains that there still exist many undetected enzymes in the salivary secretion which play a prominent role in facilitating penetration of the host tissues and hydrolysis of cell contents. The composition of saliva, and the amount secreted, differ from species to species. This indicates an intimate co-relation between characteristics of the host and enzymatic activity of the parasite. The enzymatic composition has been reported to differ even within the individuals of same species, and also at different stages of their development (Myuge, 1964).

Apart from enzymes, substances like aminoacids, amines, proteins, urea and aldehydes are also secreted by plant nematodes (Myuge, 1963). Consequent to hydrolysis of plant protein complexes by nematode enzymes, certain adverse reactions occur due to release of auxin like substances in some cases and toxins in others. Yu and Vigilerchio (1964) demonstrated that the nematodes themselves conain auxins. In roots, infected with root-nematodes, activation of gall formation has been attributed to indoleacetic acid (Anon, 1970). Studies covering formation of galls in tomato roots by *Meloidogyne* spp. revealed that instead of indoleacetic acid, indole derivatives (indoleacetoethyl ether, indoleacetonitrite and indolebutyric acid) were involved in gall formation (Decker, 1981). Jones and Northcote (1972) observed that the cell changes induced by some endoparasites result in transfer cells comparable with those normally produced in plants and the type of the eell induced differs with the genus of the namatode and in no uncertain terms is the outcome of prolonged co-evolution between the host plant and parasite.

Ecology

Earlier, ecology was limited to seeking explanations for apparently simple relationship between plant species their habitats. However, during the past fifty years advances made in studies on the effects of froral and faunal diversity movement, climate, weather, soil conditions etc. on nematodes fully establish the extent to which ecology has become indispensable to crop production and protection.

Studies on the movement of nematodes, using artificial soils and moisture characteristics, were explored by Wallace (1958). He demonstrated that there exists relation between the size of soil particles and movement of the nematodes. The larvae of *H. schachtii*, which are half in length as compared to those of *Ditylenchus dipsaci*, can move through the pores between the soil particles of the size 150 to 250 μ, while the latter cannot. This accounts for the wide distribution of *G. rostochiensis* in regions with sandy soil. Moreover, sandy soil with a better porosity is conducive to better biological activity as demonstrated by increased reproductive activity of nematodes in sandy soil (Reinmuth, 1929). Low porosity of soil is reported to suppress nematode population (Fidler and Bevan, 1963). The division of soil into light and heavy is arbitrary; none-the-less, certain species of the genera *Trophurus*, *Pratylenchus etc.* prefer heavy soil (Maeseneer, 1963).

Besides space and its configuration, the other factors influencing nematode activity are temperature, humidity, pH value, aeration, organic matter, microflora and microfauna in the soil etc. These factors act either independently or in unison with one another to influence nematode activity.

Probably, of all the factors influencing nematode activity, effect of temperature on nematodes has been extensively studied. The abundance and wide distribution of nematode species in tropical and subtropical regions is suggestive of their affinity for temperature. Eggs and encysted larvae are more resistant to vagaries temperature than the free larvae and adults (Engleman, 1943). In some species, the 2nd and 4th stage larvae are more tolerant to temperature. At lower and upper limits of temperature, breeding, development and other activities of the nematodes cease and differ from species to species (Rhode and Jenkins, 1957). Even members of the same species but of different origin react differently to temperature (Daulton and Nusban, 1961).

Necessary humidity, besides facilitating the nematode movement in the soil and on plant, is essential for longevity and survival of nematodes. The nematodes usually become quiescent when the soil becomes deficient in moisture. On return of favourable humid conditions, they again become active. Working with *Circonemoides xenoplax*, at humidity ranging between 7.8% to 17.5%, Seshadri (1965) demonstrated manifold rise in population density of this species.

The poor response of nematodes towards light accounts for absence in them of organs sensitive to light. However, light does indirectly play a significant role in nematode activity through its effect on material changes in the host plant. It is suspected that light, the long wave component of the light spectrum, accelerates the developmental processes. Short photoperiods have been reported to change the normal sex ratio in nematodes (Ellenby, 1958; Kampfe and Kersten, 1964). In studies with ultra violet rays, it has been observed that they have damaging effect on nematodes (Green and Webster, 1966; Myuge, 1965).

Conclusive inferences are difficult to arrive at from limited studies on the role of pH *vis a vis* nematode activity. Some of the studies (Godfrey and Hagans, 1933; Mai and Harrison, 1959) suggest that pH values, barring extremes, exert on appreciable influence on nematode activity. However, observations contrary to these also have been put forward. An inter-relationship between pH values and incidence of *Globodera avenae* in soil has been demonstrated by Duggan (1963) and others.

Nematodes live in close association with a variety of micro-organisms in the soil. A variety of micro-organism have been reported to be inimical to nematodes (Jhonston, 1959; Tribe, 1979; Kerry and Crump, 1979), and it is believed that some of the micro-organisms either promote or subdue the nematode activity (Decker, 1981).

Biology

Normally, most of the plant nematodes oviposit in external surroundings, where the entire embryonic development occurs. Deviation from this norm is seen in *Globodera* and *Meloidogyne* species. In some *Globodera* species, most of the eggs are laid inside the body and a part of them in a gelatinous egg case situated behind the vulva. In *Meloidogyne* species, the eggs are laid in an egg case. After laying the eggs and with the death of the female in *Globodera* species, the outer integument of the female is transformed into a cyst where the subsequent post-embryonic development undergoes. The number of eggs laid varies from species to species and their number may vary from few to several thousands.

Embryonic development starts with the cell division leading to formation of the germinal layers, and terminating into a fully formed larva. The larva, after actively coiling and recoiling in the egg case for some time, becomes quiescent. Departure from this norm is no exception since in some of the species a certain stage of development may not occur e.g., the formation of gastrula stage in *D. dipsaci* reported by Yuksel (1960). The period for embryonic development differs from species to species, and usually extends over several days.

The mechanism of larva emerging out of the egg case has not been investigated thoroughly. However, hatching of the larva is governed by the surrounding environmental conditions. In some species, root secretion of the host-plant is believed to trigger the process of hatching, and in still others the larva comes out of the egg by rupturing it by repeatedly striking with the stylet. The first stage larva, except for the absence of reproductive organs, has all the functionary organs present in other stages.

In most of the species, the first larval ecdysis takes place inside the egg and it is the second stage larva that emerges out of the egg. Phylogenetically, though, this is the second stage larva but ontogenetically it is regarded as first stage larva (Gunther, 1967). The second stage larva of most of the species of the plant nematodes is considered to be the infective stage that attacks the plant. The second stage larva after feeding for some time increases in size, stops feeding, gradually becomes inactive and finally quiescent. After the period of quiescence, it moults into the third stage larva. Likewise, the third stage larva moults into the fourth stage larva, which in turn moults into the adult stage.

After each ecdysis, the larva grows in size, and development of reproductive organs also proceeds simultaneously during this period. At each ecdysis, the body cuticle alongwith the inner cuticle lining of the oral cavity, stylet, vagina and rectum is shed away. In consistency with the mode of parasitism, as observed in *Globodera* and *Meloidogyne* species, the larval body may thicken and change in shape.

The duration of the entire life cycle of plant nematodes varies from species to species and is governed by temperature, humidity, nutritional conditions associated with the age and nature of the different host species.

Normally, the sex ratio between males and females in bulk of the plant nematodes is 1 : 1. This ratio is seen to deviate in varying degrees in some of the species, but an extreme is seen in *Aphelenchus avenae*, in which the sex ratio is 1 :10,000. Under certain conditions, the sex ratio may tilt in favour of males (Triantaphyllou, 1960 ; Lindhart, 1961 ; Decker, 1981). In *Meloidogyne* as *iacognita*, normally more males are observed to develop at lower temperatures compared to those at higher temperetnre (David and Triantaphyllou, 1967).

Plant Parasitic Nematodes

Plant parasitic nematodes are classified in a variety of ways depending on their morphology, mode of reproduction, plant organs attacked, nature of damage done, mode of life, feeding habit etc. Based on mode of parasitism, they are divided into following groups :—

Ectoparasites

Normally, this group of nematodes pierce the epidermis, dermis and cortex with their stylets and suck out the cell contents. Occasionally, these, may enter the plant tissues. A small number of these, which primarily attack fungi and algae, are reported to attack the roots of higher plants also, and are termed as facultative ectoparasitic root nematodes. These constitute a very small percentage of soil nematodes, and are represented by a few species of the genera, *Tylenchus*, *Aphelenchus* and *Aphelenchoides*. The damage caused by these does not merit much attention, unless they may be carrying plant pathogens. Among the facultative ectoparasitic nematodes, *Aphelenchus avenae* has extensively been studied (Decker, 1962 ; Barker and Darling, 1965).

Ectoparasitic nematodes have relatively been less studied. The taxonomy, biology and ecology of many species are not yet fully investigated. The harmful effects of this group of nematods did not receive much attention in many countries, inspite of a fairly large number of these species being well known. However, unlike in other countries, in U.S.A. many species were known to damage the crops (Steiner, 1949). Interest in this group received desired attention of workers when Whitehead (1965) in U.K. demonstrated that the damage to the sugar beet crop was due to ectoparasitism by the *Longidorus*, *Trichodorus* and *Paratrichodorus* species.

Ectoparasitic plant nematodes are well represented by numerous species belonging to the genera *Dolichodorus*, *Hopolaimus*, *Rotylenchus*, *Helicotylenchus*, *Scutellonema*, *Hemicycliophora*, *Circonemoides*, *Circonema*, *Hemicirconemoides*, *Macroposthonia*, *Bakernema*, *Paratylenchus*, *Xiphinema*, *Longidorus*, *Paralongidorus* and *Trichodorus*.

Normally, most of the ectopasitic nematodes develop in the soil. Occasionally, spiral nematodes (*Helicotylenchus*, *Rotylenchus*, *Hopolaimus* and *Scutellonema* spp.) burrow completely inside the root, where the entire life cycle is completed. Otherwise, they puncture the root tissue with their strong stylets and bury the anterior end of the body in roots.

Most of the ectoparasitic forms (*Rotylenchus*, *Tylenchorhynchus*, *Hopolaimus*, *Helicotylenchus etc.*) are capable of movements in the soil and infest the root system to suck out the cell contents of the root-cells with their long and stout stylets. The sapping out of the cell contents leads to death of cells fed upon and necrosis of the adjacant cells culminating in collapse of the tissue. Stubby root nematodes (*Trichodorus*) and dagger nematodes (*Xiphinema*) are typical ectoparasites and usually feed on root caps, root hairs or cells immediately behind root caps. As a result of damage to actively growing parts, the growth of the roots is retarded and quite often the infested roots collapse. Besides, swellings resembling galls may form on infested parts of the root and the portion behind such swellings usually necroses.

There are few ectoparasitic leaf nematodes (*Aphelenchoides*, *Ditylenchus* spp.) which feed on surface of the leaves in developing leaf buds or flower buds. The strawberry nematode (*Aphelenchoides fragariae*) lives both ectoparasitically or endoparasitically depending on the host species.

Semi-endoparasites

Probably, no clear demarcation can be drawn between the ectoparasitic and semi-endoparasitic nematodes. However, there are certain species *Rotylenchus reniformis*, *R. borealis*, *Tylenchulus semipenetrans*) which penetrate the anterior part of their body into the root to suck nutrients. The posterior portion that remains outside the roots becomes highly swollen. The saccate females loose mobility and become sedentary.

Endoparasites

Nematobes of this group invade the plant tissues, and live there. They are known to infest both underground and aerial parts of the plant. Among these, those parasitising the roots are of extreme economic importance and have relatively been more studied. The more important genera of endoparasites come under the families Heteroderidae, Tylenchulidae, Hopolaimidae, Circonematidae and Tylenchidae, and are repesented by the genera *Heterodera*, *Globodera*, *Meloidogyne*, *Meloidera*, *Tylenchulus*, *Hyposoperine*, *Sphaeronema*, *Trophonema*, *Rotylenchus*, *Nacobbus*, *Cacoparus*, and *Anguina*. These are sedantary in nature and include cyst forming, gall forming and non-cyst and non-gall forming nematodes. The members of the genera *Pratylenchus* and *Pratylenchoides* are migratory in nature and endoparasitic on roots.

Among the endoparasitic root nematodes, the cyst and gall forming nematodes constitute a highly specialised group. In cyst forming nematodes, *Globodera* species, the females usually lay most of the eggs inside the body. However, some of these species besides laying eggs inside the body also lay a part of eggs in a gelatinous egg case situated behind vulva. After cessation of oviposition, the female dies and its outer body integument gradually hardens into a leathery cyst. The formaticn of cyst is an adaptation against the adverse effect of the conditions prevailing around. The cysts vary in size and shape and are an aid in identification of a few species.

The infective larva of the cyst forming nematodes enter the meristem of the plant immediately behind the root tip and lie parallel to the axis of the root tip with anterior end away from the root tip. The symptoms and nature of damage to the host plant varies from species to species. In the potato nematode, *Globodera rostochiensis*, the sucking of nutrients and mechanical injury during penetration and formation of cyst by females leads to stems becoming fragile, leaves looking etiolated and reduced in size and conduction of food and nutrients to the upper parts of the plant impaired. In *H. schachatii* the sugar beet nematode, the infestation results in stunting of primary root and to over come the effects of stunting of the primary root, many rootlets keep developing.

Root galls and thickenings resembling root galls are formed by many species of the genera, *Meloidogyne*, *Nacobbus* and *Anguina*. *Meloidogyne* species are known to form typical root galls. The second stage larvae of the *Meloidogyne* species, in the soil, puncture the young roots of the host plants immediately behind the root tips. After gaining entry, the larvae move to the root cortex and lodge themselves parallel to the longitudinal axis of the root to remain in close proximity of the conducting system of roots. The subsequent feeding, developmental and reproductive processes continue here. To the salivary exudate of the juveniles and adults, the hosts react by forming giant cells around the cephalic end of the different parasitic stages. This is accomplished by dissolution of the cell walls of the cortical cells and the vascular tissues of the roots. Hypertrophy and hyperplasia of the cortical cells lead to the formation of galls. The galls vary in size and shape depending upon the parasite, the host plant, the soil type and other factors.

The non-cyst or non-gall forming endoparasitic species of roots belong to the genera *Pratylenchus*, *Pratylenchoides*, *Hirschmanniella* and *Radopholus*. The larvae and adults of these species are actively motile. The different stages attack the young roots a little behind their tips to enter the cortex and feed on its cell contents. Having entered once, the parasites usually move along the longitudinal axis of the root, leave the tissue, and re-enter the same or adjacant roots of the same or the other plant. Because of their migratory nature, both juveniles and adults are found in immediate surroundings around roots. Eggs are usually laid in rows along the route the females traverse. and entire development takes place in side the tissues of the host plant

The *Ditylenchus* species are typical endoparasites of the stems, leaves, tubers, bulbs, corms, rhizomes and even seeds. The infective stage usually enters through stomata and with the setting in of infestation the middle lamella of the cell wall dissolves. The weakened celluar structure leads to formation of conspicuous cavities aud rapid multiplication of cells in the area, which results in thickening of the tissues. The feeding, developmental and reproductive processes take place within the tissues of the host.

The leaves, buds and flowers are attacked by some *Aphelenchoides* species. The infective stage of the *Aphelenchoides* species enters the leaf through the stomata and lives in the intercellular spaces of the mesophyll tissue. As a result of feeding on cell contents, the chloroplast disappears and the cell walls dissolve to give rise to large cavities in the infested tissue. Consequently, the tissue collapses. Some of the *Anguina* species are also known to parasitise leaves and floral buds. The parasitisation leads to formation of galls. In cereals, parasitised spikes give rise to galls instead of seeds.

Though nematodes have been classified as ectoparasitic, semi-endoparasitic and endoparasitic; many species lead both ectoparaitic and endoparasitic mode of life.

The above account is limited to only few important genera of plant nematodes.

Nematodes and Plant Pathogens

The punctures and lesions formed by nematodes, in roots and other plant organs, remain open to infection by plant pathogens. Disease symptoms like stunted growth, chlorosis, necrosis and ulceration, die-back, root-rot etc., produced by viruses, bacteria and fungi, could also be attributed to nematodes. This overlapping of disease symptoms makes correct diagnosis of the disease problematic. The relationship between nematode parasites, pathogens and host-plants is so complex and specialised that the disease symptoms may be absent or masked.

It is well established that plant nematodes act both as carriers and vectors of plant pathogens. Experimental transmission of a plant pathogenic virus was first demonstrated by Hewit, Raski and Goheen (1958). Transmission studies by a number of workers have revealed that the prime vectors of plant viruses are limited to the order Dorylaimida. Both, the larva and adult are capable of acting as carriers or vectors of viruses. Studies show that *Xiphinema* and *Longidorus* species are associated with viruses which are spherical, and *Trochodorus* species are associated with the rod shaped viruses. This nature of association is attributed to structural and physiological differences between the species of the aforementioned genera.

Viruses usually remain virulent for several weeks and under certain circumstances for considerably longer periods (Taylor & Raski, 1964). However, viruses gradually loose their virulence with the passage of time. The larvae have been reported to loose their infectivity after ecdysis. This loss of infectivity is attributed to shedding of the inner lining of the cuticle at the cephalic end (Harrison and Winslow, 1961). Studies are yet to establish transovarial transmission, and multiplication of viruses within the nematode body (Decker, 1981).

Various species of namatodes have been incriminated as vectors of many plant pathogenic bacteria. Most of the namatode species associated with bacterial diseases belong to the genera *Meloidogyne*, *Helicotylenchus*, *Pratylenchus*, *Aphelenchus*, *Aphelenchoides*, *Ditylenchus* and *Anguina* (Kalinenko, 1936 ; Cheo, 1946 ; Steward and Schindler, 1956 ; Hawn, 1965 ; Libman, Leach & Adams, 1964). Apart from acting as vectors, nematode species are known to break down the natural resistance of the host plants and render them susceptible to bacterial infection (Lucas, Sasser & Kelman, 1954).

The relationship of the nematode species of the genera *Globodera*, *Meloidogyne*, *Pratylenchus* *Radopholus*, *Tylenchorhynchus*, *Rotylenchus*, *Ditylenchus*, *Anguina* and *Belonaimus* to different pathogenic fungi is well essablished (Hastings and Boscher, 1938 ; Neal 1954 ; Newhall, 1958 ; Gill, 1958 Myuge, 1959 ; Taylor and Wyllie, 1959 ; Blake, 1961). The natural resistance of plants breaks down as a result of nematode infestation and they become susceptible to fungal attack. The susceptibility of tubers to the attack of *Phytophthora* infestation and onion bulbs to 'crown rot' by *Botrylies allii* is attributed to the attack by *Ditylenchus destructor* and *D. dispasci*, respectively. Myuge (1959) attributes the break down of natural resistance in plants to the adverse enzymatic activity of the salivary exudates released by the parasitic nematodes. Plants resistants to fungi or nematode parasites respond differently to the combined nematode-fungus infection (Jenkin and Coursen, 1957; Leach, 1958; Taylor and Wyllie, 1959; Mountain and McKeen, 1962; Ross, 1965).

Control Strategies

Primarily, the control of plant parasitic nematodes in infested areas is beset with difficulties due to the huge amount of the top soil and water in it. Secondarily, the numerous species of plant parasitic nematodes with varied ecology, biology, host-range, mode of life etc. render the control operations all the more difficult. Consequently, a wide range of cultural practices and control procedures are employed to keep their population at such a low level that their presence is of no significance. Some of these measures include chemical control (including fumigation); biological control; breeding of resistant varieties; crop rotation; companion planting; changes in time of sowing; organic manuring; using artificial fertilizers; soil treatments; fallowing; flooding of land; application of gamma rays; ultraviolet rays, electricity and chemosterilants; steaming and cleansing of seed stocks and soaking of plant stocks.

For maintenance of physical features and nutrient level of the soil, crop-rotation has been in practice since Roman times. Coupled with these beneficial effects, crop-rotation also contributes towards keeping the weeds, diseases, pests and parasites at a tolerable level. Therefore, botanically diverse crop rotation can effectively control such plant parasitic nematodes which are relatively plant specific and immobile. After withdrawal of the host-crop, a period of 4–5 years does not eliminate the parasite but reduces the population to such a low level that they do not effect the crop yield. This feature of crop rotation has been successfully exploited in Europe to have profitable yields from fields infested with gall forming nematodes of potato (Decker, 1981). However, crop-rotation becomes problematic in case of nematodes with a wide range of hosts.

It has been demonstrated that the root exudates, released by different species of plants, vary in chemical composition (Sayre, Patrick and Thorpe, 1964). In certain cases, the root exudate secreted by a particular plant may stimulate hatching of the cysts of a specific parasite but may prove toxic to other. This is well illustrated in case of polyphagous stubby root nematode, *Trichodorus christie*, which rapidly multiplies on tomato roots but fails to do so on asparagus roots (Rhode & Jenkins, 1958). The root exudate released from the asparagus plant contains a glycoside that reduces the population of the stubby root nematode of tomato to such a level where damage to the crop is almost insignificant. With the accumulation of our knowledge on numerous naturally occurring compounds in plants which afford protection to them against the attack by various pathogens (Swain, 1977) and the advantages of companion sowing (Putman and Luke, 1978), the concept of companion planting is bound to find favour with scientists and agriculturists.

A series of changes occuring in the soil due to ploughing, harrowing, hoeing, irrigation etc., usually do not bring down the population of parasitic nematodes to such a level that they become incapable of diminishing the crop yield. However, ploughing of soil has been reported to adversely affect the nematode populations in the soil (Oostenbrink, 1964).

The use of fertilizers, primarily used for increasing the soil fertility, is reported to control plant nematodes, Free stages of the nematodes exhibit susceptibility to the nematicidal action of fertilizers, but no adverse effect has been observed on eggs and encysted stages. Dressing of the sandy soil (with high pH) with slaked lime resulted in impressive decrease of free stages of the nematode genera *Globodera*, *Trichodorus*, *Hemicycliophora* and *Pratylenchus* but the encysted forms remaind unaffected (Kuper and Leeuw, 1963). De Groote (1960) reported effective control of the endoparasitic and ectoparasitic species of the genera *Pratylenchus* and *Hopolaimus* with a spray of calcium cyanamide at a rate of 12 q/ha. On the contrary, Edwards (1937) observed that calcium cyanamide, even at very high doses, is ineffective in controlling *H. schachtii*. Studies on the effect of fertilizers on plant nematodes reveal that the action of different types of fertilizers differs from species to species (Curtis, 1964; Vassalo, 1965).

It is reported that organic and green manuring, in addition to promoting plant growth, inhibit the pathogenecity of plant nematodes by suppressing their population, retarding larval development, conferring resistance to plants and accelerating the deleterious activity of natural enemies of plant nematodes (Koltermann, 1943; Ellanby, 1945; Van der Laan, 1965; Duddington, 1957; Egunjobi and Larinde, 1975). Both, organic and green manures of different origin are reported to considerably differ in their composition; therefore, they have varied action on different species of nematodes.

Very few studies have been conducted to assess the effect of microelements (manganese, boron, magnesium, molybdenum, etc.)on plant nematodes. The available information is contradictory and of little importance to derive any significant inference.

Numerous studies have established that among various factors governing nematode development, edaphic temperature is most important. Changes in temperature and and various edaphic factors can be corporated by advancing or delaying the sowing or planting time of certain crops. By corporating changes in sowing time of potato, significant decrease was noticed in the population of the cyst-nematode, *Globodera rostochiensis* (Kradel, 1958 ; Brande and D'Herde, 1964). However, before corporating any changes in sowing or planting time of a crop, it should be ensured that the change does not affect the crop yield adversely.

Breeding of plant varieties resistant to nematode infestation is extremely important and beneficial in as much as it can be extended to all crops and has long range advantages at a comparatively low cost. Effective screening of plants for resistance, nature and source of resistance, and manipulation or recombination of resistance with all the indispensable features of a cultivar are prime requisites for successful breeding of resistant plant varieties. Usually, difficulties crop up when the genetic change is not limited to the host-plant alone, but also extends to the nematode. In certain types of resistances, selection for resistant plant varieties is countered by those variants in the nematode parasite that are the result of simultaneously ongoing process of natural selection in the nematode. In cases of this nature, success for evolving a resistant variety against a parasite depends on the rate of change induced in the host-plant by breeder and rate of change in the pathogenicity of the parasite while undergoing natural selection. Primarily, on a wide genetic basis, there are two main types of resistances ; one in which the genes for resistance in the host-plant are matched by genes for pathogenecity in the parasite and the second, where there is no such matching i.e. the genes for resistance in the host-plant and the genes for pathogenicity in the parasite have no direct relation.

Inspite of its vast and complex nature, the breeding of plants is being extensively studied and resistant cultivars for many crops have been raised (Birchfield and Jones, 1961 ; Evans and Stone, 1977 ; Lim and Castillo, 1979).

A variety of soil organisms, antagonistic to soil nematodes, are either parasitic or predatory. The parasitic organisms are represented by viruses, bacteria, protozoa and fungi. The predatory forms being nematodes, turbellarians, enchytraeids, insects, mites and fungi. The predatory or parasitic nature of various organisms has been exploited to control parasitic plant nematodes biologically. The literature on the biological control of nematodes has been reviewed from time to time (Sayre, 1971 ; Webster, 1972 ; Sterling and Manaku, 1977).

Studies, using natural enemies, were first conducted by Linford and Oliveria (1937) followed by Duddington (1951) and many others. The initial hopes raised for successful control of nematodes with the nematode trapping fungi (Duddington, 1956) did not last long since the nematode trapping fungi failed to give reasonable control of nematode populations. Recent studies have demonstrated that *Paecilomyces lilacinus*, a parasite on eggs of *Meloidogyne incognita*, lowers production of viable eggs by 70% (Jatala, Kaltenbach and Bocangel, 1979). Studies on biological control of *H. avenae*, cyst nematode of cereals, by the parasitic fungi *Nematophthora gynophila* and *Verticillium chlamydosporium* revealed that 60% of the females failed to form cysts containing eggs, more so in case of *N. gynophila* (Kerry, Crump and Mullen, 1982 a,b). Many other studies of similar nature suggest that deployment of biological control agents for suppression of nematode population is a feasible proposition in as much as no environmental pollution or toxic hazards are involved in their application. However, their success as biological control agents will depend on their adaptability to the surroundings, mobility, host-specificity, ability to find the host, survival in the absence of host and the timing of their activities with those of the host.

Among various control methods in practice for suppressing populations of plant parasitic nematodes in soil, the use of a wide range of chemicals is most effective and practical. The cuticle of the juvenile and adult stages of nematodes offers resistance to entry of nematicides. The eggs and encysted larvae offer still greater resistance. On adults and free larval stages, the nematicide acts by entering through the oral, anal and other openings in their body. In addition to resistance offered by nematode cuticle, the nematicidal activity of various chemicals is also influenced by various factors like-the physical and chemical properties of the nematicide, soil temperature, soil moisture, organic matter present in the soil, soil type, presence or absence of host plants and mode of application.

The nematicides, besides acting against the nematodes, also act against other soil organisms. The destruction of natural soil microflora and microfauna result in upsetting of the biological equilibrium in the soil. Many nematicides (methyl bromide, chloropicrin *etc.*) ; also known as soil-sterilants or disinfectants, also possess bactericidal and fungicidal properties. Consequently, application of such nematicides leads to killing of many benificial soil organisms (i.e. natural enemies of nematodes). This makes the choice of an efficient nematicide problematic. However, the populations of soil micro-organisms builds up rapidly to restore its original level. Moreover, there is no evidence to suggest that with the destruction of natural enemies of nematodes, the nematode population flares up. Inspite of this, care has to be exercised in the use and choice of a nematicide to see that biological control agents also contribute fully towards natural control of nematodes.

Kuhn (1881) is credited for being first to use a chemical, carbon disulphide, for the control of sugar beet nematode (*H. schachtii*). This was followed by the use of calcium cyanamide for control of root-knot nematode of tobacco. The use of some of the present day soil fumigants as effective nematicides was reported by Mathews (1920), Taylor and McBeth (1940), Carter (1943) and Christie (1945). The contribution of the above and few others prompted a number of workers to screen a wide range of chemicals for their nematicidal properties. Consequently, spectacular progress has resulted in the area of chemical control of plant nematodes (Taylor, 1965 ; Webster, 1972 ; Whitehead, 1975 ; Berkum and Hoestra, 1979). The search for potent, safe and economical nematicide will continue to remain an ongoing process till the end has been achieved.

After 1945, numerous chemicals have been tested for their nematicidal properties. These nematicide include organic halides, organophosphates, carbamates, isothiocyanates, sulphur compounds, mercurials, cadmium compounds, selenium compounds, copper compounds, arsenic compounds, aziridine derivates (chemosterilants) and a host of others. Currently, excluding the combination of nematicides (vortex, nemax, dorlon etc.), the nematicides available in the world market are:— aldicarb, carbofuran, chloropicrin, dichloropropane and dichloropropene mixture (DD), ethylenedibromide, ethotrop, phenamiphos phensulphothion, methyl bromide and oxamyl (Anon, 1984). One of the highly effective and relatively less phyto-toxic compound, dibromochloropropane, has recently been withdrawn because people involved in its manufacture became sterile.

The different groups of compounds with nematicidal properties are broadly categorised into soil-fumigants and non-fumigants. The soil-fumigants (organic halides and methyl isothiocyanate liberators etc.) are applied to the soil as liquids, emulsions, powders and granules. The active ingradient of the soil-fumigants, or soil-fumigants as such, transform into the gaseous form. The time required to transform into gaseous form differs for different soil-fumigants, and is greatly influenced by the soil temperature and soil moisture besides other factors. The soil-fumigants on being evenly applied to the soil diffuse in all directions. The gaseous vapours cover the soil-particles, soil-organisms and the underground parts of the plant, and under suitable conditions combine with the thin film of water surrounding the soil particles and soil organisms to form a strong solution. The organic halides (methyl bromide, dibromochloropropane, dibromoethylene etc.) represent the old group of soil-fumigants, while vapam, vortex, dazomet etc. represent the new group liberating isothiocyanates. The soil-fumigants are extensively used against several species infesting a wide range of crops (Hannon, 1964; O'Bannon and Bistline, 1969, Jones, 1970; Hawkins and Miller, 1971).

Non-fumigant nematicides include a wide range of compounds which are water soluble. These are represented by organophosphates, carbamates and others. Some of these function either as contact nematicides (phensulphothion, ethoprop etc.) or systemic nematicides (maleic hydrazide, sodium fluroacetate, dizinon, dimethoate, phorate etc.). Still others (aldicarb, carbofuran, phenamiphos etc.) act both as contact and systemic insecticides. The non-fumigant nematicides have been reported to act against cyst, root-knot lesion, ring and other nematodes (Whitehead, 1973; Minton and Morgan, 1974; Chabra and Mahajan, 1976; Bordie, 1980). Systemic nematicides, when applied directly on plant or in the soil, enter the plant and are carried to different parts of the plant by the conducting system of the plant. In recent years many non-fumigant nematicides, such as oximecarbamates and organophosphates have been tested for control of several species of nematodes. These studies have revealed that non-fumigant substances act in a variety of ways to control nematodes (Stendel, 1972; Steel and Hodges, 1975; Griffin, 1975; Batterby, 1979). The problem of phytotoxicity is not acute with most of the non-fumigant insecticides. However, many of the non-fumigants are highly toxic to man and animals and persist in the environment for long periods. Therefore, more and more constraints are being put on their use. The recent tragedy, which took toll of more than 2,000 lives in Bhopal, India, is bound to have its repercussions on the use of nematicides.

Very few studies have been conducted on the mode of action of different groups of nematicides in nematodes, and the problem of resistance to nematicides in plant nematodes has not been encountered yet.

Conclusion

The global existence and the damage done by plant nematodes to almost every type of vegetation and field crops has tremendously increased man's concern for their control. Apart from morphological studies, supplementary information from varied disciplines like physiology, biochemistry, genetics etc. has helped in better understanding of the taxonomy and phylogenetic relationship within the phylum Nematoda. However, the existing taxonomic grouping of nematodes is yet not altogether satisfactory. Moreover, the diagnosis and account of different taxa are yet insufficient. Correct diagnosis of species, races or biotypes is essential before any physical, cultural, biological or chemical measure can be institued for controlling any of the plant nematodes. Therefore, with newer techniques and innovations in optics at our disposal, revisional studies on nematode biology, behaviour, biochemistry, physiology and genetics etc. are indicated. Such studies are bound to be helpful in scanning vulnerable points in the nematode life-histories for devising more effective control methods.

With more and more constraints being put on the use of chemical nematicides, in view of their toxicity to man and animals and their persistence in the environment, search for safer chemical nematicides is to be intensified. Search for such insecticides needs to be extended to many plant species known to possess nematicidal properties. To minimise the use of chemical nematicides, an effective strategy involving combined application of cultural, physical, chemical and biological methods has to be investigated vigourously.

In text books on invertebrate Zoology, the plant nematodes have either been treated superificially, or do not find any place. Therefore, keeping the importance of plant parasitic nematodes in view it is desirable that plant nematodes get adequate representation in text books on invertebrates and teaching programmes for graduate and post-graduate students.

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7

HUMANKIND AND BIOSPHERE

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Introduction

Man, in ecological perspective, is a domninant species of the environment. It is rather impossible to separate man from the biosphere of which he forms an integral part. The air he breathes, the water he drinks, the sunlight that warms him, and the food he eats, all tie him up to his immediate physical and biological environment. At every time the human body, like that of any other organism, exists in a dynamic state of exchange with the environment-taking in or giving out gases, absorbing or giving off heat, losing or gaining water and other chemicals; and this continuous process of exchange ceases only with death. But before becoming a dominant species in the environment, man lived as a member of a biotic community without having any more effect on the environment than any other animal species would have. In other word, man and nature (*i.e. Purush* and *Prakriti*) existed in perfect harmony with each other and formed an inseparable part of the life-support system. Now. however, with the advent of man as a maker of new intricate tools and as a procedure of food, new relationships have been introduced into the environment, which has started to transform and to become dominated by human skill. In another way, we could say that the environment has started changing from a system-in-itself into a resource for one of its component species, *Homo sapiens*. This raised many problems for the environment and for the human race of today. This article deals with the man-biosphere interactions and the possible causes of global environmental problems.

Biosphere and its Components

The term 'biosphere' was first used by the Austrian geologist *Eduard Suess* in 1875. The concept was further developed by the Russian mineralogist *V I. Vernadsky*[1] who published his two lectures first in Russian in 1926 and later in French in 1929 under the title 'La Biosphere' Biosphere means the sphere of life or the envelope of life. It has been defined by *Hotchinson*[2] as that part of the earth in which life exists. However. this definition raises some problems because the life also exists above the earth's surface in the form of "aeroplankton." Thus, the largest system in which a large number of natural and human ecosystems can operate together is known as the biosphere. The three components of the biosphere are :*atmosphere*, *lithosphere* and *hydrosphere* and these are inter-related through the continuous process of energy flow and cycling of materials (fig. 1). These three subdivisions of the biosphere are controlled by natural phenomena that are beyond the manipulations of man. Living organisms require inorganic metabolite from each of them. The *atmosphere* supplies oxygen, nitrogen, and carbon dioxide: the *lithosphere* supplies all the necessary minerals; and the *hydrosphere* supplies water. Together, these inorganic materials provide all the chemical elements needed in the formation and maintenance of living matter (Fig. 2). In addition to being everlasting sources of supply, they are the media that may receive wastes and effluents of human activities, and influence metabolic functions of living organisms in various ways. It would not be out of place to describe the role of these components of the biosphere.

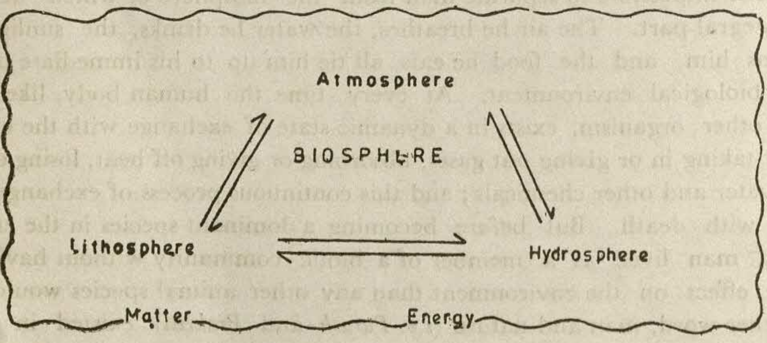

Fig. 1. The three components of the biosphere

Atmosphere

The atmosphere is the gaseous component of the biosphere. It is usually divided into four layers called the *troposphere*, *stratosphere*, *mesosphere*, and *thermosphere*. The lowest 10 km of the atmosphere (the troposphere) has an especially significant role in the biosphere. It controls the earth's weather and receives and disperses practically all the airborne emissions of modern civilization.

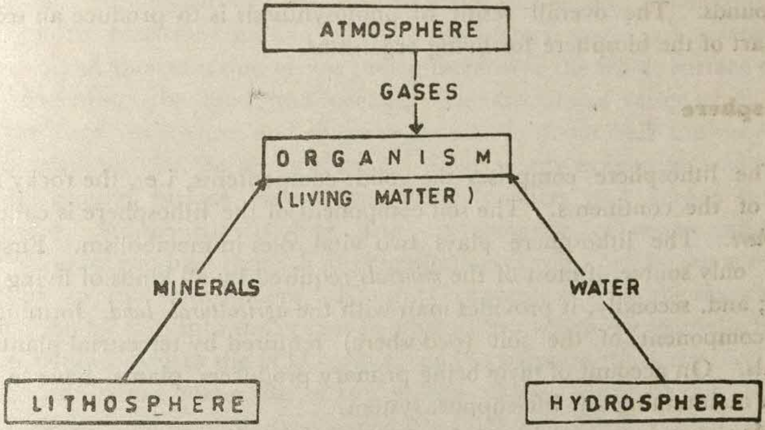

Fig. 2. The material contributions of each of the three subdivisions of the biosphere towards maintenance of living matter

Changes in the atmosphere may have profound effects on the organisms on earth, on local weather patterns, and on regional and global climatic patterns. For example, increase in atmospheric carbon dioxide has a green house (warming) effect and could lead to an increase in air and water-temperatures and, hence, to an increase in evaporation and changes in rainfall patterns. Particulates emitted into the atmosphere during the combustion of fossil fuels increase the atmospheric turbidity. Small particulates remain in the atmosphere for a longer time and undergo chemical reactions to produce secondary air polluntants. Sulphur oxides and nitrogen oxides enter the atmosphere through burning fossil fuels and industrial emissions. Acid rain, caused by sulphuric and nitric acids that results from sulphur and nitrogen oxides collecting in the atmosphere, has become a matter of serious concern in Europe and North America. Acid rain has caused ecological damage to vegetation and soil. It has also caused the impoverishment of freshweter lakes, especially those over igneous rocks as as in eastern Canada and Scandinavia. An increase in atmospheric radiation also causes harmful effects. These few examples are sufficient to illustrate that changes in the atmosphere, caused mainly by man-made emissions are not only responsible for air pollution but have far reaching effect on the boisphere.

Of still greater significance to metabolism are the chemical cycles of the atmosphere. The air consipts mainly of oxygen, carbon dioxide, nitrogen, water vapour, and minute traces of inert gases. Except the inert gases, all theses components of air serve as matabolites and each circulates through a cycle in which living organisms play a prominent role. The ecological importance of the oxygen, carbon dioxide, and nitrogen cycles is well known. No doubt the present energetics of the boisphere depends on the use of carbon dioxide in photosynthesis to form molecular oxygen and organic compounds. The overall result of photosynthesis is to produce an oxygen-rich part of the biosphere for living organisms.

Lithosphere

The lithosphere comprises the solid compontents, i.e., the rocky substance of the continents. The soil component of the lithosphere is called the ***pedosphere***. The lithosphere plays two vital roles in metabolism. Firstly, it is the only source of most of the *minerals* required by all kinds of living organisms; and. secondly, it provides man with the *agricultural land*, forming the bulk component of the soil (pedoshere) required by terrestrial plants and animals. On account of their being primary producers, plants have a vital role in maintaining our life-support system.

Man's various activities also influence the functioning of lithosphere. The composition and condition of agricultural soil are influenced greatly by farmers. They may add chemical fertilizers to improve soil fertility, or unknowingly, they may do things that degrade it in some way. In modern agriculture fertilizers like nitrates and phosphates are used. A considerable amount is washed off the agricultural fields through irrigation. rainfall, and drainage into rivers, ponds and lakes where they may seriously disturb the aquatic ecosystem. Excessive use of fertilizers often leads to accumulation of nitrates in water which cause human health problems· A major source of pesticides in rivers,·streams and lakes is from runoff from agricultural fields. Some pesticides also enter inland waters from industries which use pesticides in their manufacturing proceses. Insecticides like DDT are known to exert their adverse effect though biological concentration are considered hazardous pollutants of soil and water.

As stated above the greatest contributions of the lithosphere are the minerals and the agricultural land. But only 13.4% of the world's total land space is suitable for food production. This limited proportion underlines the importance of conserving our croplands under an ingenious land-use policy. But at the same time the questions like "can we produce enough food for world population", and "how many people can biosphere support without impairment of its overall functioning" demand serious thought and should be answered carefully. Answers to similar questions and to the functioning of the biosphere have been nicely described by *Woodwell*[3], *Brown*[4], *Singer*[5], and *Brown*[6].

Hydrosphere

Life also exists in small bodies of water and in lakes, rivers, streams, estuaries, and seas. Together, they form the hydrosphere. Water covers about 73% of the earth's surface and is one of the essential requirements of life.

The global *water cycle* is a continuous process of evaporation, transpiration, precipitation surface run-off, and ground water movement. About 507 Tm^3 (1 tetra cubic metter=1 million million cubic meters) water are evaporated each year, and the same quantity is precipitated over the whole surface of the earth, including the land and oceans, The amount of water which flows from the land in streams and rivers to the seas is about 44.5 Tm^3 per years and is available for the needs of man. Solar energy evaporates water from the soil, ground surface, vegetation, water surfaces and oceans into the atmosphere. Subsequent cooling and condensation of water vapour at higher altitudes produce clouds and precipitation as rain, hail or snow returns the water to the hydrosphere Natural evaporation from the seas exceeds precipitation by rain into the oceans by about 9%, which is eventually moved as water vapour over to the land surface, and so balances the water cycle and provides additional water for man's needs. Thus, water as rain, hail, or snow is precipitated over the land and water surfaces. Water on land surface eventually percolates into the soil as soil or ground water. Some of the ground water that is absorbed by plants passes out of the leaf surfaces by transpiration, which is an important process for conduction of water and dissolved minerals throughout the plant. Thus, through the natural process of hydrological cycle water is exchanged between atmosphere, land, sea, all living organisms.

In using water as a metabolic raw material, organisms withdraw it mainly from the hydrosphere. Aquatic animals absorb water directly from their environment and excrete some of it back while alive, and after death the remaining water is returned through decay. Terrestrial animals absorb water from the soil and also from freshwater bodies. Animals and plants move such water through their bodies and in the process retain the required amounts. The rest is excreted, partly as liquid water and more as water vapour which naturally raises the moisture content of the atmosphere. Thus, a given amount of environmental water goes from hydrosphere to atmosphere more rapidly through the metabolic process of living organisms than if that water were simply allowed to evaporate directly from the hydrosphere. In other words, the metabolism of terrestrial organisms actively contributes towards the maintenance of the global hydrological cycle.

Water influences metabolism not only as the most important nutrient but also through its effects on climate and weather, both in the sea and land. The oceanic currents influence climatic conditions not only within the seas but also in the air and on land. Another climatic effect is the result of the thermal properties of water. The global climate is also influenced by the amount of water locked into polar ice. All these cyclic changes in the hydrosphere have profound impact on metabolism. By influencing temperature, humidity, amount of precipitation, winds, waves, currents, and indeed the very presence or absence of water in given localities, they play a major role in determining the kinds of organisms that may exist at a given time on land and in water and air.

In using water for different needs and in exploiting water resources for aquaculture, man has made considerable changes in natural water bodies. Water is polluted by sewage and domestic wastes industrial wastes and effluents, insecticides, heavy metals, and radioactive nuclides. Man has not spared even the holy rivers which have already lost their pristine wholesomeness. Fortunately, the monitoring and control of water pollution have now been taken up by all the countries and the supply of potable water is an important issue before all governments. The most significant consequence of human waste disposal in waterways is the accelerated eutrophication of lakes. How the man-made changes in the water bodies and man's other activities affect the natural aquatic communities, may be understood by examples like the fastly depleting freshwater fishes (*Singh* and *Badola*[7],[8]). The famous mahseer, which was so abundant in Kumaon lakes has now completely disappeared from Nainital lake (*Das* and *Pandey*)[9]. Man-made changes in the rivers and streams are adversely affecting the spawning behaviour of freshwater fishes. Transportation of timber in rivers kills Innumerable eggs of desirable fish species (*Badola* and *Singh*)[10]. These few examples from recent studies indicate that man has done irreparable loss to the natural water bodies.

Biogeochemical Cycles

In the above description of the biosphere we have seen that the chemical elements tend to circulate in the biosphere in characteristic paths from environment to organisms and back to the environment. These more or less circular paths of the chemical elements are known as *biogeochemical cycles.* In this term "bio" refers to living organisms and "geo" to the rocks, soil, air and water of the earth. According to *Odum*[11], *biogeochemistry* is the study of exchange of materials between living and nonliving components of the biosphere. For each cycle two compartments or pools may be recognised. These: (a) the *nutrient pool* or the *reservoir pool*, which is the large, slow-moving generally nonbiological component; and (b) the *exchange* or *cycling pool*, which is a small but more active portion that is exchanged.

There are two basic types of biogeochemical cycles *gaseous* and *sedimentary types*. In the former, the atmosphere constitutes the major reservoir of the element which exists there in the gaseous state. The *carbon dioxide*, *oxygen* and *nitrcgen* cycles are good examples of such biogeochemical cycles. In the sedimentary type of cycle the major reservoir is the lithosphere, from which the elements are released in course of time by weathering. Among others, the *phosphorus* and *sulphur* cycles are excellent examples of sedimentary types of cycles.

The presence of nitrogen and its compounds is essential for the maintenance of life in the biosphere. However, atmospheric nitrogen, which is about 79% of air, cannot be used as such by the majority of organisms. It must be "fixed" by specialized organisms or by industrial processes.

The most common usable nitrogen source is the *nitrate* ion (NO_3), which may be used by plants as a mineral metabolite from the environment and may be ultimately converted by them into proteins. Nitrates are added to the environment (Fig. 3) through fertilisers, dissolution of rock by water, combination of atmospheric nitrogen with oxygen by lightning (nitrates so formed reach the soil by rain), and through the process of *nitrification* as shown below:

$$NH_3 + 1\,{}^1/_2 0_2 \text{———} H_2 0 + HN0_2 \text{ (Nitrous acid)}$$
$$KN0_2 + {}^1/_2 0_2 \text{———} Kn0_3 \text{ (Potassium nitrate)}$$

In the nitrogen cycle the organic nitrogen of plants and animals is ultimately broken down by soil microbes to ammonia (NH_3) in a process called *ammonification.*

$$CH_2\,NH_2\,COOH + 1\,{}^1/_2 O_2 \text{———} 2C0_2 + H_2 0 + NH_3$$

Some of the ammonia is oxidized to from *nitrites* ($N0_2$) and *nitrates* ($N0_3$) through the action of chemotrophic bacteria, e.g., *Nitrosomanas* and *Nitrobacter.* This process is called nitrification and the energy released supports existence of these bacteria. Other types of soil bacteria, e.g., *pseudomonas denitrificans* are able to use the nitrate ion for the oxidation of organic compounds, a process which is called *denitrification,* by which N_2 is liberated into the atmosphere. Denitrificatian reactions are shown as follows:

$$C_6H_{12}O_6 + 6KNO_3 - 6CO_2 + 3H_2O + 6KOH + 3N_2O$$
$$5C_6H_{12}O_6 + 24KNO_3 - 30CO_2 + 18H_2O + 24KOH + 12N_2$$
$$5S + 6KNO_3 + 2CaCO_3 - 3K_2SO_4 + 2CaSO_4 + 2CO_2 + 3N_2$$

Nitrogen is removed from the air by the nitrogen-fixing bacteria, which live either freely in the soil (saprophytic bacteria like *A zotobacter* and *Clostridium*) or as symbionts (symbiotic or nodule bacteria like *Rhizobium*) in the root nodules of legumes. Thus, by converting aerial nitrogen into usable nitrogen, the nitrogen fixing bacteria complete the nitrogen cycle. Reactions for nitrogen fixation are as follows:

$$N_2 \text{———} 2N \text{ (activation of nitrogen)}$$
$$2N + 3H_2 \text{———} 2NH_3 \text{ (actual fixaton)}$$

Before nitrogen can be fixed it must be "activated", which means that molecular nirogen must be split into two atoms of free nitrogen. This step requires an investment of energy (about 160 kilocalories for each mole

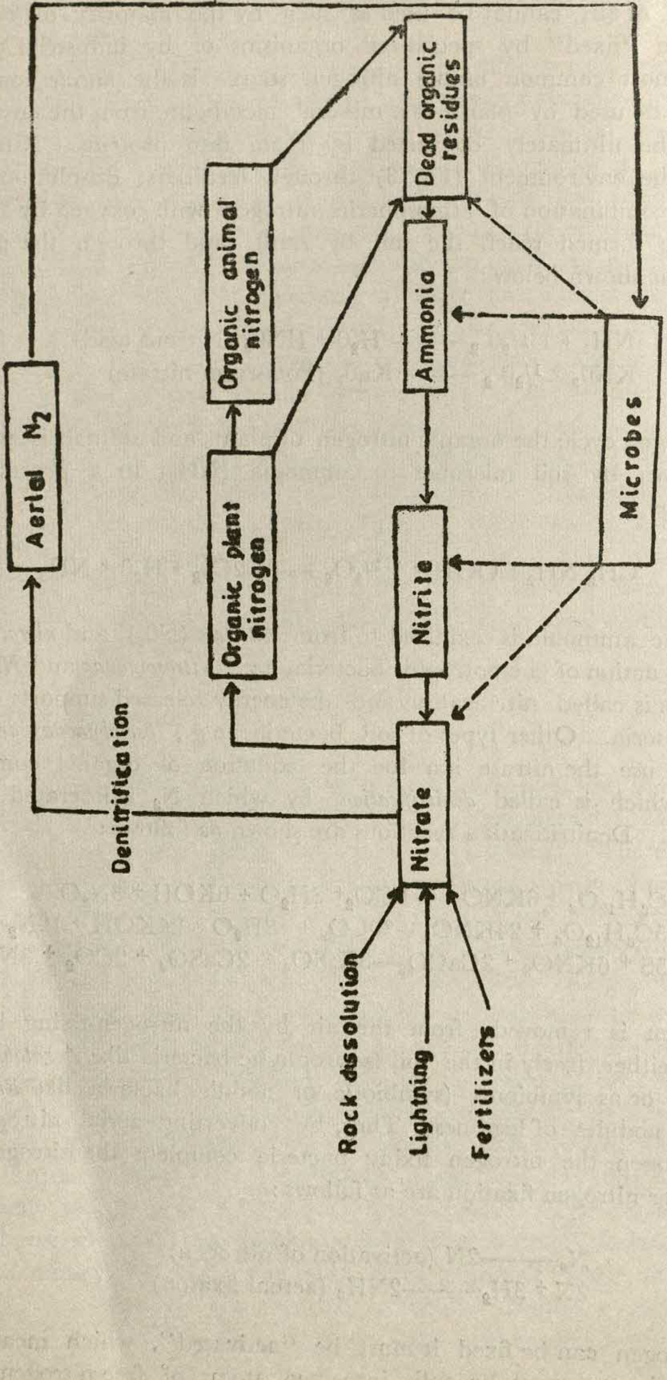

Fig. 3. The nitrogen cycle

of nitrogen). The acual fixation step, in which two atoms of nitrogen combine with three molecules of hydrogen to form two molecules of ammonia (NH_3), releases about 13 kilocalories. Thus, the two steps together require a net input of at least 147 kilocalories. Once ammonia or ammonium ion has appeared in the soil it can be absorbed by the roots of plants and the nitrogen can be incorporated into proteins. As plant is eaten by an animal, the nitrogen may be incorporated into a new protein. The death and excretion of organisms ultimately return proteins to the soil, where it is decomposed by soil microbes which utilize nitrogen compounds for their metabolism. As proteins are decomposed by these bacteria, a chain of intermediate compounds such as ammonia, nitrites, and finally nitrates is produced. Nitrates are absorbed by plants and so re-enter the nitrogen cycle. Through the natural cycle the concentration of nitrogen in the atmosphere is kept relatively constant. In modern agriculture nitrogenous fertilizers are added to the soil and this practice, no doubt, upsets the balance of the natural cycle. Pollution of water is taking place by the leaching of excessive amounts of nitrate from agricultural land into lakes, rivers and streams.

Phosphorus Cycle

The basic source of phosphorus are the rocks formed in past geological ages. Terrestrial plants absorb inorganic phosphates from the soil (Fig. 4a) and convert them into organic phosphates. Animals obtain their phosphates from plants. Phosphates absorbed from the soil are returned to it in the form of dead plant and animal organic residues, which are converted to humus by soil microbes. In the soil, much of the phosphate is fixed or absorbed on to soil particles, but some is lost thraugh leaching out into waterways.

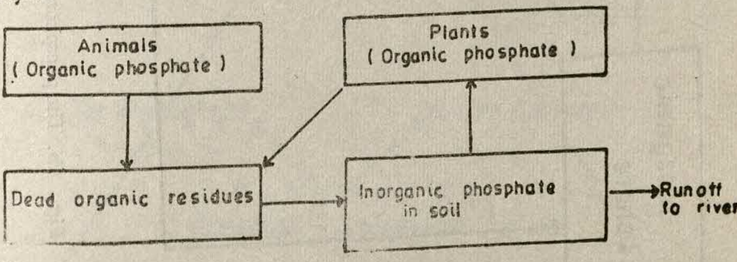

Fig. 4a. The phosphorus cycle in soil

In water, phytoplankton absorb soluble inorganic phosphates (Fig. 4b) and convert them into organophosphates. Phytoplankton provide food for zooplankton which, in turn, are consumed by other animals. After the death of animals, the organic debris decays through the action of microbes and phosphates are released into the water for recycling.

Dix[12] has described that the natural phosphorus cycle can be substan-

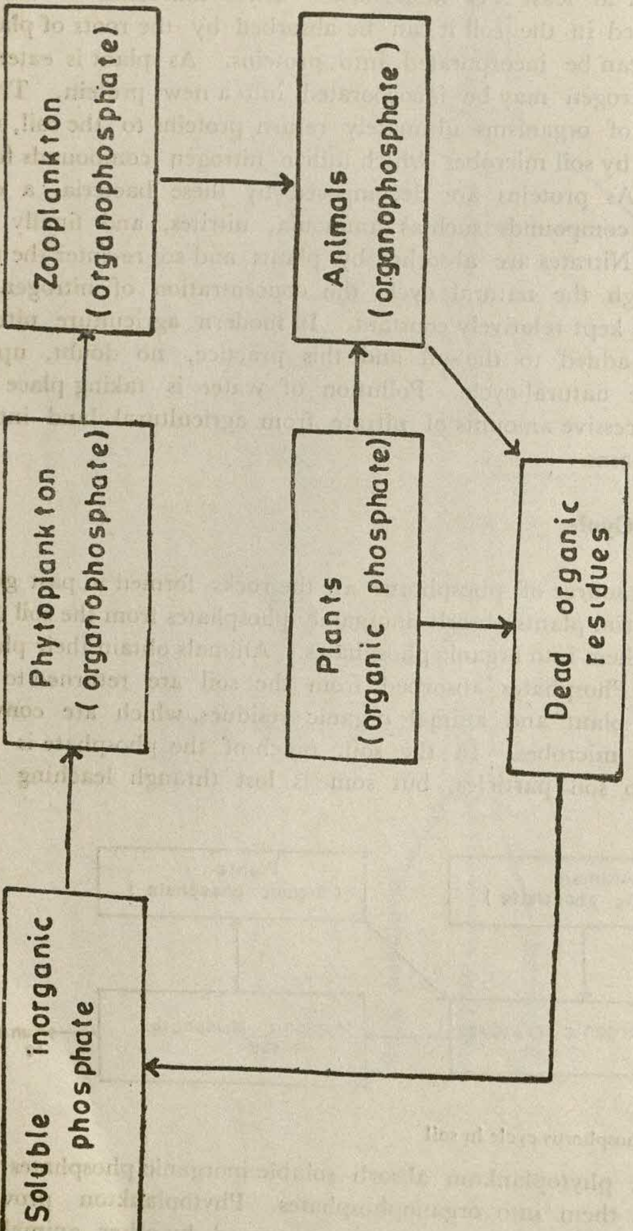
Soluble inorganic phosphate
Phytoplankton (organophosphate)
Zooplankton (organophosphate)
Plants (organic phosphate)
Animals (organophosphate)
Dead organic residues

Fig. 4b. The phosphorus cycle in water

tially affected by pollution. Superphosphate and triple superphosphate are now frequently used on land in agriculture. Sewage, even after treatment, contains phosphates derived from excreta and detergents. These phosphates can ultimately reach freshwater rivers through land run-off and effluent discharge. In the U. K., it has been estimated that $2x10^5$ tonnes of phosphate snters sewers each year, and $1.32x10^5$ tonnes or 66% of this is discharged into rivers. Phosphate pollution of lakes and rivers has caused excessive growth of algae, which depletes the dissolved oxygen content and disrupts the natural food chains (Dix, 1981).

Man-Biosphere Interactions

The advent of mankind and his eventual prominence has created new dimensions in the functioning of the biosphere. As man distinguished himself amongst the biotic components of the biosphere as *'special'* or *'dominants,'* his role had a gradual impact on many natural phenomena, causing :, for example, the extinction of thousands of desirable species of plants and animals, changing natural vegetation over vast areas of land and modifying the global water cycle through worldwide river controls. According to *Kassas,*[13] the man-biosphere interactions can best be conceived as inter-relationships among three systems: the ***biosphere technosphere*** and ***sociosphere.*** It may be mentioned that the biological, socio-cultural and technological parts of man's life-support system depend on the biosphere (Fig. 5).

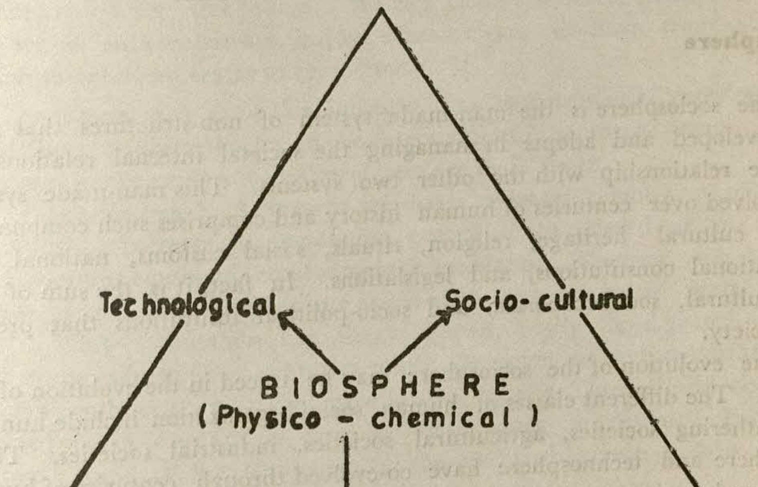

Fig. 5. Relationship between biological, socio-cultural and technological parts of man's life-support system and their dependence on biosphere.

Biosphere

The biosphere is the largest natural system including atmosphere, lithosphere and hydrosphere, and the living biota of these components. This system has far longer history than makind, and its operations follow global and universal phenomena that are beyond the control of man at the macro level. It provides man, animals and plants with the *space* to live, and the *elements* that man develops into resources for his needs.

Technosphere

The technosphere is the man-made system of structures that man prepares and sets for his use within the space of the biosphere. It includes the rural and urban settlements, industrial centres, transport and communication structures, irrigation, drainage, and river control systems, hydro-electric and atomic energy centres, farmlands and their machinery, modern warfare equipments, and so on.

The evolution of the technosphere from its simplest to the ever increasing complex form has been directed and governed by human skill. Therefore, this artficial system is largely under the control of man who has been shaping and modifying this system to suit his changing needs. However, few components of the technosphere, such as the farming systems though managed by man, are not under his full control. They are still under the influence of natural processes like climate and rainfall.

Sociosphere

The sociosphere is the man-made system of non-structures that man has developed and adopts in managing the societal internal relationships and the relationship with the other two systems. This man-made system has evolved over centuries of human history and comprises such components as the cultural heritage, religion, rituals, social customs, national and international constitutions, and legislations. In fact, it is the sum of the socio-cultural, socio-economic, and socio-political institutions that prevail in a society.

The evolution of the sociosphere may be traced in the evolution of the society. The different classes of human social organization include hunting and gathering societies, agricultural societies, industrial societies. Thus, sociosphere and technosphere have co-evolved through centuries of human history and are inter-connected, inter-related and inter-dependent. Whenever the society has pushed back the limits imposed by the environment, it has been either through substitution of one resource for another e.g., fossil fuels for wood, animal power for human power, machine power for animal power, vegetable protein for animal protein, and the like, or understanding a specific part of the system in a better way so that it could be used more effectively e.g., using the existing genetic reservoir within crop plants to develop high yield varieties through a scientific breeding programme).

The interactions between society and the biosphere components include the patterns by which people alter them and the effects of those changes on society Resources result from interactions between the three systems societal action on natural elements of the biosphere, using the tools of the technosphere. *Kassas*[13] has stated that the elements of tne biosphere remain as geological formations, hydrological processes or biological organs until (i) man discovers their utility in meeting needs and requirements; (ii) man develops means of obtaining, and treating if necessary, these elements; and (iii) man transforms the elements into resources.

Barry Commoner's "*first law of ecology*" (*Commoner*[14]), which states that "*everything is connected to everything else*," helps us in understanding the complexity of multidimensional interactions among the bisophere, tehchnosphere and sociosphere (Fig. 6). No doubt, a developed society is one that has the capability for managing the complex interrelationships between the three systems, and an under-developed society is short of this capability. A developed society can decide how to manage its landscape, but it cannot determine the environmetal principles that govern the responses of the landscape to that management; nor can it choose how others manage theirs (*Clapham, Jr.*[15]). For example, we can choose to have wilderness (untamed nature) or to cut virgin trees, but we cannot choose that soil will not erode after tree felling. We can choose to have cities that dump toxic wastes into waterways, but we cannot choose to have trout still swim through the polluted water to reproduce.

The above two examples indicate that environmental problems arise from failure, or discrepancies, in the interactions of the three systems. Some *direct causes* of the environmental problems are as follows:

(i) *Population growth*: At present we have a world population of about 4. 6 billion. Increase in population growth is considered as one of the most important causes of environmental problems. However, the rate of increase has not been constant, nor has it been the same in all parts of the world. While population has always grown, really rapid growth did not begin in the industrialized countries until the onset of industrialization in the eighteenth century. The growth rate of the Third World countries remained fairly constant as long as these areas represented backward societies. And, at the beginning of the twentieth century, their rate of growth began to rise sharply.

(ii) *Industrial growth*: Industrial plants are established on the space provided mainly by the lithosphere. As industries use the elements and the raw materials from the biosphere, and throw the waste products into the environment, they play an important role in environmental pollution and degradation.

(iii) *Agricultural chemicals*: Farming is the main food-producing system.

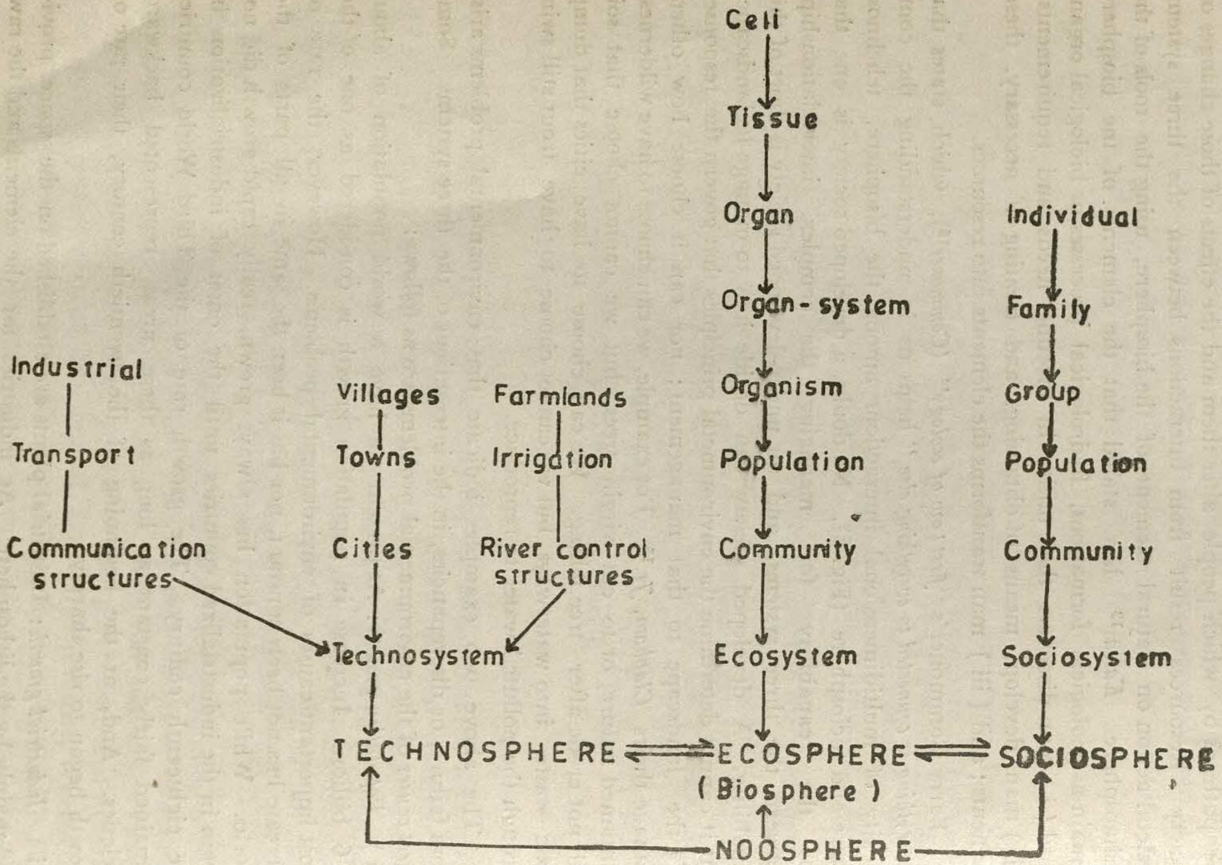

Fig. 6. Relations between the biosphere, technosphere, and sociosphere and the role of noosphere

It also provides a variety of other products. Fertilizers, so commonly used in modern agriculture, and residues of pesticides and herbicides create a number of environmental impacts in air, soil and water

(iv) *Energy generation*: Energy is a critical requisite of any society. In a global sense, there are only three ultimate sources of energy — the sun, gravity, and the atomic nucleus. Population, industrial and agricultural growth all depend on energy and in his efforts of more and more energy generation for various needs man uses the meterials from the biosphere and, in turn, creates a number of environmental problems. The problems associated with the nuclear energy transformation (radioactive emissions, neuclear fission, and nuclear fusion) are both frightening and exciting.

(v) *Deforestation*: Forests are one of the most imprtant natural resources. The most significant contribution of a forest is to provide man and other living organisms with oxygen. Man has been cutting for various needs. Deforestation creates problems not only in one component of the biosphere which sustains them (the lithosphere), but in due course of time the other two components are also seriously affected. When forests are cut down, not only their biota is affected, but soil is eroded, land is degraded, ground water channels are altered, and water becomes polluted and scarce.

(vi) *Overexploitatioon of resources*: Nature has given us a number of resources — water, minerals, flora, fauna, and so on. But our faulty methods and policies of resource utilization are responsible for most of the environmental problems. Due to lack of ecological awareness, man considers that resources are 'inexhaustible.' Overexploitation of that forest resource has already created an ecological imbalance in many parts of the world and especially in the Himalayas. Overexploitation of fishery resource has alrerdy reduced the number of desirable fish species like the cod, trout, and mahseer. Resource expoloitation and pollution are interrelated phenomena; since the former at one place may cause the latter at the same or other place. For example, fishing by the use of dynamite, ichthyotoxic plants, and chemicals, also pollute the environment (Singh and Badola[7]).

(vii) *Defective policies*: Many environmental problems arise due to defective planning and pollicies. Policies with regard to city-planning, river-control, forest-use, land-use, and other similar policies, have been awefully defective and have gone a long way in creating and developing a number of environmetal problems.

Of the above direct causes or *human activities* that are responsible for the creation, development and increase of envioronmental problems, all are important. But their impact at any given time has not been the same in different parts of the world. In most of the cases the known causes of environmental problems have been interrelated. For example, a defective forest-use policy encourages overexploitation of forest resource, resulting in deforstation which, in turn, creates serious environmental problems. Deforestation in the upper catchement area of the Bhagirathi is considered the root cause of the famous *Bhagirathi blockade.* The blockade, formed in

August 1978 due to a huge landslide in to the Bhagirathi near Gangnani, resulted in unforeseen changes in the Bhagirathi ecosystem and when it was cleared the water washed off abont 60 thousand trees, 12 bridges, a large number of telephone poles and houses, and many kilometers of road. The fish fauna of the Bhagirathi, effected by blockade, has not recovered as yet (*Sharma* and *Singh*[16]).

We know that due to defective land-use policy agricultural land is being reduced day by day. This worldwide process presents an example of failure in the relationship between the sociosphere and the biosphere. The area of land available to man is about 134 million Km^2, of which only 18 million Km^2 or 13. 4% is used by the food-producing system. This limited proportion underlines the importance of conserving the agricultural land. But in developing countries millions of hectares of productive land are taken over by the technosphere. The average annual losses of agricultural land due to four main processes : non-agricultural use, erosion, desertification and toxification have deen calculated to be 8, 3, 2, and 2 million hectare respectively. Losses due to erosion, desertification and toxification are mostly due to failure in the relationship between the technosphere (means used in land management) and the biosphere (resilience of the soil system) *Kassas*[13].

In many instances the constraints of the sociosphere are standing in the way of man to cope with changes in the biosphere and with advancements of the technosphere. Due to such reasons man is unable to make demographic adjustmets between densely populated areas.

The Role of Noosphere

It has been suggested that the man-biosphere relationship is influenced by a fourth system called the *noosphere* (*noos* meaning mind). In fact it is the sum of all the knowledge that mankind applies to build up the technosphere and sociosphere, and to manage the man-biosphere relationship.

The term '*noosphere*' was first used by *Vernadsky Vernedsky*[17] in a classic paper entitled 'The biosphere and the noosphere'. The noosphere concept was intended to represent a final stage in the evolution of biosphere. By noosphere Vernadsky meant 'the sphere of mind' that was to supersede the biosphere, the sphere of life. "*We live in a transition to the noosophere*" these words of Vernadsky clearly explain that the mankind as a dominant species in the biosphere has started transforming into the noosphere.

The noosphere has considerably disrupted the man-biosphere relationship, due to which the very existence of man is in peril. Today 'the living together' relationship between the humankind and the nature, that existed so well in ancient times, is no more seen. Bary Commoners' "third law ecology" (*Commoner*[14]), which states that "nature knows best" is an eye-opener for all of us. It holds that the natural relationsphips that have emerged after 3.5 billion years of evolution are at least the best available model for human behaviour and may constitute the only valid model for managing the biosphere. Therefore, if a choice has to be made between a natural way and an artificial way of dealing with an environmental problem, the natural way should be chosen and environmental planning should be based on a broad view so that a healthy environment may be generated.

Acknowledgement

This article is partly based on the lecture delivered by me at the Department of Zoology, Lucknow University, Lucknow on April 5, 1984. I am grateful to Professor Bhoomittra Dev for providing a stimulating interaction during the lecture and for his valauble suggestions.

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8

PHYSIOLOGY OF THE NEURONE

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Introduction

Cells (neurones) capable of responding to the stimuli by generating electric currents (signals) appeared first in the coelenterates. At the initial stages of evolution, the neurones assembled to form a simple nervous system in which they were organized in lose network. In the network the nerve cells could communicate with each other but the flow of signals was generally multidirectional and poorly channelized. Activities of the organism could be controlled by the nerve nets without a central control. Gradually with the march of evolution, complex nervous systems composed of organized dense clusters of neurones came into being. A great variety of morphological types of the neurones evolved; their physiological specializations and the molecular mechanisms they employed to carry out the task of conducting, transmitting and integrating the information became increasingly complex and fascinating. In a complex nervous system (e. g. vertebrate central nervous system), a nerve cell is a component of an assembly of a large number of interacting nerve cells. For example, in a nervous system made up of 10^{11} neurones (higher mammalian nervous system), each neurone may have approximately as many as 10^4 interconnections [10]. The interaction (communication) between the neurones is always through speciallized contact points called synapses which are the sites of intense and complex physico-chemical activity (Fig. 1). All of the neurones comprising a nervous system are not grouped into one constellation but are organized into a collection of a large number of distinct even morphologically distinguishable constellations (many a times each one of which may be made up of a variety of morphologically and physiologically distinguishable characteristic cell types for example cerebral cortex, cerebellar cortex, reticular formation etc.) which extensively interact among themselves. The aggregates (constellations) of neurones may have a high degree of physiological specialization. For example in mammalian brain, initiation of activity in certain aggregate (s) of nerve cells may cause the onset of sleep (raphe nuclei) while the activity in another (locus eeruleus) may cause dreaming and the activity in others will initiate waksng state. Still further, continued activity in other organized structures (reticular formation) would be necessary to maintain alertness and waking state. In the brain of humans, particular groups of neurones in the left cerebral hemisphere cortex take care of the functions of comprehension of language, making grammatically correct sentences, act of speech etc. Complexity in the structural make up and physiological specialization of the neuronal aggregates have been attained through a long process of evolution during which stage by stage newer and newer, and more and more complex efficient circuitory and biochemical and physiological processes were acquired. Study of the phylogeny of acquisition of physiological specialization and competence by various brain structures (parts) and of addition of new parts has made it possible to understand to a great extent as to how mammalian brain in general and human brain in particular has come to possess extremely complex physiological, psychological and behavioural characteristics.

The nervous systems have become equipped to receive channelized information through a variety of receptors (sense organs), to process it, to use it and also to create new information[130]. They have also acquired capacity to store and retrieve information, and to control and coordinate the functions of other organ systems. The nervous system came to play a major role in making the organism aware of its internal and external environments and thus in helping the organism to adjust and adapt to the internal and external changes. The neurone being the unit of structure and function of the nervous system and also as a distinct cell type continues to be an exciting challenge for biologist and non-biologists alike. For instance, a neurone is capable of generating demonstrable electric potential differences and electric currents and using them for processing, transmitting, integrating and conducting information over long distances. The nerve cells through a series of interactions can compute (like a computer) the information and provide a remarkable output. The electrical potential fluctuations are phenomena not divorced from metabolic activities and a complex series of molecular mechanisms underlie them and as such they are an integral part of the living activity of the cell. It has been said that manifestations of life are not conceivable without molecular (chemical) changes[85],[87], and the electrical phenomena can also be considered as the expression of the living activity of the neurones; sometimes electrical potential changes themselves can maintain and induce relevant chemical events on which the living state of the cells depends.

It is commonly known that the nerve cells as well as the muscle cells are post mitotics because they do not continue to divide throughout life and are thus not renewable. Once a full complement of nerve cells has been established, not only they do not proliferate but also their number may decrease with time. Age-related changes leading to senscence probably may best express themselves in the tissues comprised of postmitotic cells. The neurones, thus, are of particular interest from the standpoint of gerontology[105],[107]. As the nervous system has a control over all other body systems, it is always of key interest. Even actvities such as meditation (practised by yogis) seem to have some interesting effects on nervous system functioning as judged through electroencephlography[133].

Even one single neurone in itself is a complex organisation. For the sake of simplicity a neurone is pictured as having a cell body (perikaryon) from which emerge a large number of processes the dendrites and one long fibre like process, the axon, which at its tip arborizes into terminal branches that establish contact with other neurones. This simple scheme shows many variations when one considers the exact morphological pattern of particular type of a neurone in space. For instance, a Purkinje cell of the cerebellar cortex is characteristically different in morphological features from a pyramidal cell of the cerebral cortex. Many more such examples can be cited. Physiologically the neurones can be classified into sensory, inter-and motor neurones. These distinctions are important when a neurone is to be considered as one of the members of a system in which the role of a neurone becomes specified : a sensory neurone would serve its primay task of conducting and transmitting information from the sensory (receptor) cells and a motor neurone of suppling the information to the effector cells such as muscle cells. The interneourones may be interposed between the two and can increase the anatomical and physiological complexity of the concerned input-output pathway. Physiological events associated with a neurone include receiving inputs, from several other neurones, integration (computation) of these inputs, generation of its own response and finally conducting and transmitting it to the other neurones or effector cells. Regions for these functions are spatially distict in the cell (Fig. 1). In this article, my attempt is mainly to focus attention on, and survey, physiological mechanisms underlying signal production and transmission in the nerve fibres.

Some Characteristics of the Neurone

A neurone in its entirety is bound by the excitable plasma membrane (neurolemma). In a nerve cell the plasma membrane is referred to as excitable because of its ability of being excited to generate signals that code information, and is functionally distinguishable from non-excitable ones. The excitation process (signal generation) is considered to be mostly a membrane phenomenon. It is thus of considerable importance and interest to consider various physiological and biochemical events that occur in the membrane and the role they play in excitation of the neurone.

Most cells generate an electrical potential difference across their surface membrane. This potential differnce is caused by a differential distribution of cations and anions on the outside (extracellular surface) and the inside (cytoplasmic surface) of the membrane. A measurable potential difference is detectable in all kinds of cells including plant cells. Nerve cells and muscle cells are the examples of excitable cells as distinguished from most other cells which can be regarded as nonexcitable cells. The membrane potential in an excitable cell is capable of being purturbed by stimuli in a manner that results in the generation of measurable and propagatable signals possessing codes derived from stimuli. It is not out of place here to mention a few examples of non-nervous cells which besides exhibiting the property of excitability also produce electrical signals somewhat similar to those in nerve cells *Amoeba* and *Paramecium* are organisms (protozoa) structured as one cell. *Amoeba* cell has a membrane potential difference across its plasma membrane, which is negative (–80 mv) with respect to the surrounding. This membrane potential is depolarized by K^+ and also varies spontaneously producing spike-like potentials mediated by an inflow of Ca^{2+} into the cell through the localised membrane area where permeability to calcium increases[86a] *Paramecium* is

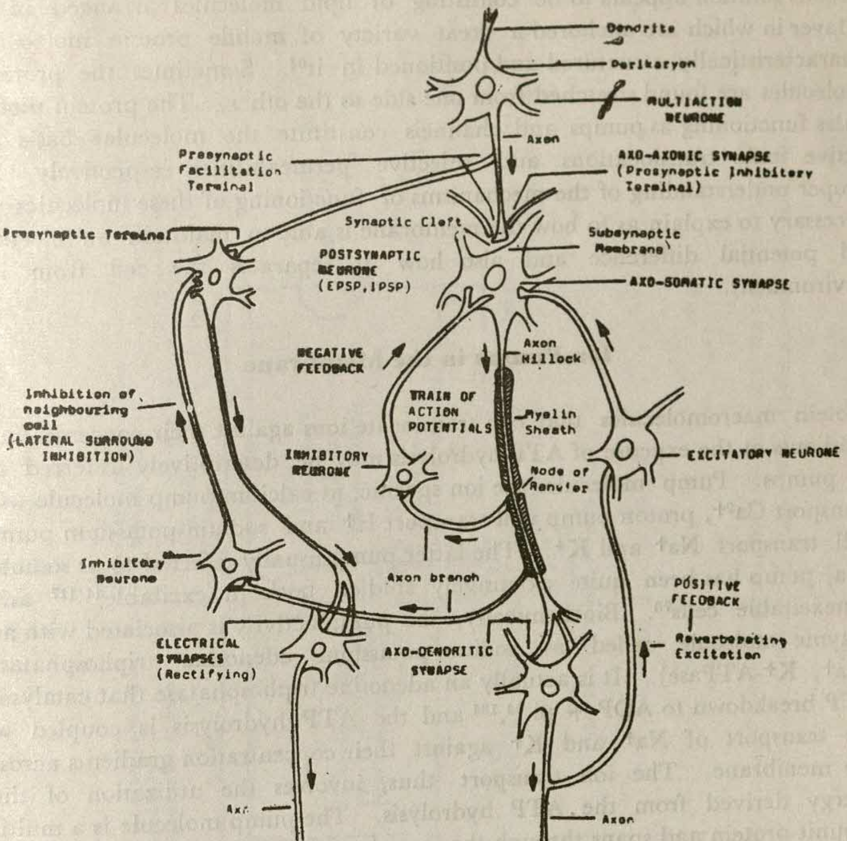

FIGURE 1

Fig. 1. Diagramatic repesentation of some of the several ways in which neurones may come in contact with and interact each other. All of these features may not be present in the circuits of one cell group. Note multiaction neurone, positive and negative feedback circuits (reverberating excitation for learning), lateral surround inhibition, electrical rectifying synapse.

another interesting example that merits attention as an excitable cell. The ciliary membrane in this organism responds by producing Ca^{2+} action potential which controls motor responses of the organisms [46], [86]$_{2}$a [49]. In plant kingdon, excitable cells are found in *Mimoso pudica* [‘03], *Nitella* and *Chara*[53].

The membrane potential difference is a basic electrical characteristic of the plasma membrane and should be adequately understood for proper elucidation of the mechanisms underlying excitation and conduction. Membrane may be considered as a 2-dimensional viscous solution surrounding the cells. It acts as a barrier with selective permeabilities. Chemically, the viscous solution appears to be consisting of lipid molecules arranged in a bilayer in which are anchored a great variety of mobile protein molecules characteristically structured and positioned in it[64]. Sometimes the protein molecules are found stretched from one side to the other. The protein molecules functioning as pumps and channels constitute the molecular basis of active ionic translocations and selective permeabilities respectively. A proper understanding of the mechanisms of functioning of these molecules is necessary to explain as to how the membrane is able to maintain an electrical potential difference and also how it separates the cell from its environment.

Ion Pumps in the Membrane

Protein macromolecules that can translocate ions against their concentration gradients at the expense of ATP hydrolysis may be descriptively referred to as pumps. Pump molecules are ion specific, as calcium pump molecule will transport Ca^{2+}, proton pump will transport H^+ and sodium-potassium pump will transport Na^+ and K^+. The latter pump usually referred to as sodium (Na) pump has been quite extensively studied both in excitable[44,117] and nonexeitable cells[70]. Biochemically, the pump activity is associated with an enzyme molecule called as sodium-potassium adenosine triphosphatase (Na^+, K^+-ATPase). It is actually an adenosine triphosphatase that catalyses ATP breakdown to ADP + pi [54,134] and the ATP hydrolysis is coupled to the transport of Na^+ and K^+ against their concentration gradients across the membrane. The ion transport thus, involves the utilization of the energy derived from the ATP hydrolysis. The pump molecule is a multi-subunit protein and spans through the membrane. It is asymmetric in its transmembrane orientation since at the intracellular side it carries catalytic (ATP-hydrolysing) and Na^+-binding sites whereas on the extracellular side it carries the K^+-binding site and also a site specifically sensitive to a pharmacological agent ouabain. The mechanism by which Na^+, K^+-ATPase mediates metabolically driven vectorial transport of the ions up and down the membrane, actively in either direction, is not precisely known although a number of hypotheses have been proposed[44,123]. The molecule requires phospholipids for its activity and transports Na^+ and K^+ through a multiple reaction sequence[113] which involves interconvertible enzyme forms resulting from conformational changes[70]. At the Na^+-sensitive site (Fig. 2) the molecule in one state of its conformation probably picks up Na^+ and translocates it up the membrane to the extracellular surface and releases it there. At this stage the molecule undergoes a conformational change, becomes sensitive to K^+, binds K^+ from the extracellular medium, translocates it down the membrane and ultimately releases it into the cytoplasm. Alongwith the site (s) where K^+ is released and Na^+ is picked up is also located the catalytic site where ATP hydrolysis occurs. The ATP hydrolysis requires Mg^{2+} which is

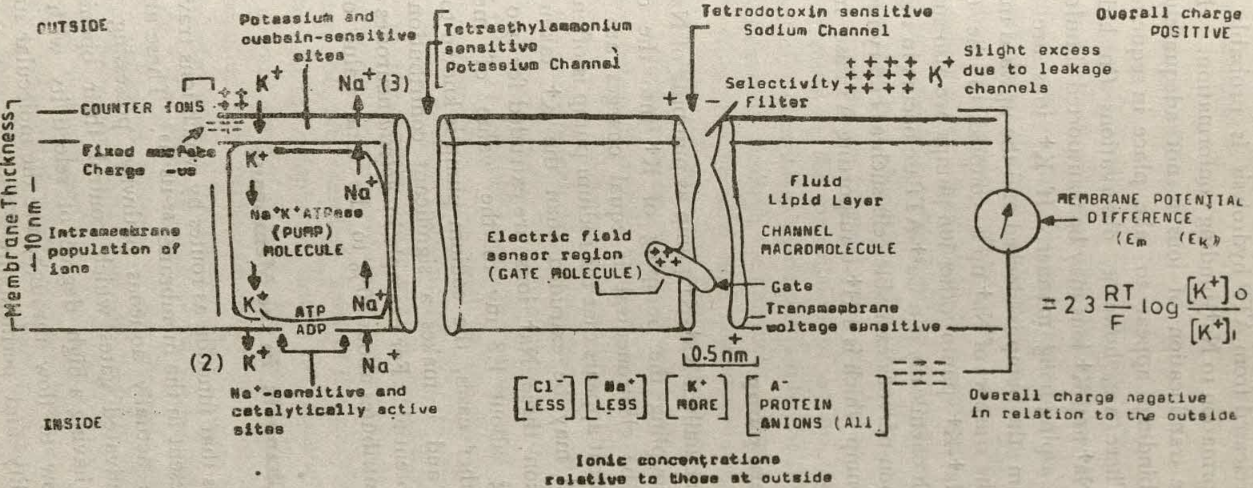
OUTSIDE
Potassium and ouabain-sensitive sites
K+
Na+ (3)
Tetraethylammonium sensitive Potassium Channel
Tetrodotoxin sensitive Sodium Channel
Overall charge POSITIVE
Slight excess due to leakage channels
Selectivity Filter
COUNTER IONS
Fixed surface Charge -ve
Membrane Thickness —10 nm—
Intramembrane population of ions
Na+K+ ATPase (PUMP) MOLECULE
ATP
ADP
Electric field sensor region (GATE MOLECULE)
Fluid Lipid Layer
CHANNEL MACROMOLECULE
Gate
Transmembrane voltage sensitive
MEMBRANE POTENTIAL DIFFERENCE (Em (EK)
$= 2 3 \frac{RT}{F} \log \frac{[K^+]_o}{[K^+]_i}$
0.5 nm
(2) K+
Na+
Na+-sensitive and catalytically active sites
INSIDE
Cl- LESS
Na+ LESS
K+ MORE
A- PROTEIN ANIONS (All
Overall charge negative in relation to the outside
Ionic concentrations relative to those at outside

FIGURE 2

Fig. 2. Schematic representation of some membrane features relevant to the understanding of ionic transport, membrane potential, active transport etc. in the axonal membrane.

not transported and it appears to occur during the period when K$^+$ dissociates to diffuse into the cell interior and Na$^+$ is picked up to be transported up the membrane. The energy released from ATP hydrolysis is probably used to convert K$^+$-binding conformation to Na$^+$-binding conformation. How ATP hydrolysis is coupled to the translocation of ions is not adequately understood[134]. Na$^+$ and K$^+$ bindings appear to take place in sequence rather than simultaneously[44]. There are, however, observations (in kidney cell pumps)[31] indicating that Na$^+$ and K$^+$ ions may be transported simultaneously rather than sequentially in a ping pong manner. If K$^+$ ions are removed from the external medium the pump is blocked and the upward movement of Na$^+$ ions falls because the trasport of Na$^+$ up is coupled to the transport of K$^+$ down. Besides Na$^+$-K$^+$ pump other ion pumps are also sufficiently known. Ca^{2+} pump which chemically is a Ca^{2+}-ATPase occurs in nerve and muscle cells and also in non-nervous excitable cells such as ciliary membrane of *Paramecium*[28]. Proton pump which is a H$^+$-transporting ATPase may also be found in cell membranes.

Electrogenicity of Sodium pump

Since Na$^+$ pump is actually a Na$^+$-K$^+$ pump as pumping of Na$^+$ out of the cell has a component coupling the pumping of K$^+$ into the cell, the coupled transport would be electroneutral if equal charges are pumped inward and outward. But it appears that the sodium pump is often electrogenic[128] as a little more Na$^+$ may be extruded than the K$^+$ taken in. It appears that in squid axon three Na$^+$ ions are extruded for two K$^+$ ions brought in[57] contributing about 1.3 mv to the value of membrane potential[27]. In several other cases, the sodium pump is known to be considerably electrogenic [24,63] and it makes a significant contribution to the value of the membrane potential. For example in *Aplysia* neurones Na$^+$-K$^+$ exchange pump activity contributes about 30% to the total value of membrane potential[16,41].

Ion Channels in the Membrane

Discrete moleculor entities that function as routes by which ions traverse the membrane passively are present in the membranes [52,118,119]. These are called ion channels. They are actually aqueous pathways possessing voltage-activated or chemically-activated gates which control their opening and closing. The ion channels having a high degree of selectivity with regard to the ions they can allow to flow through, are the molecular structures responsible for the excitability and permeability. They are the most important proteins for nerve function. Mechanism (s) underlying the passage of ions through the channels continues to be a subject of intensive investigation [4,21,52,821,19,141]. Channel molecules are protein macromolecules and as far as is known lack enzymatic activity[4]. This latter porperty and the non-involvement of high energy sources[51,52] (ATP *etc.*) in their function distinguish channel molecules as conducting pores[4,65] from the pump molecules which are carrier molecules.

Ion Selectivity of Channels

The channels exhibit a high degree of ion selectivity. The Na^+ ion-selective channel will not permit K^+ ions to pass through, and similarly the latter will not allow the former. However, it may be noted that the K^+ channel will permit thallium, rubidium and ammonium (but not Na^+); the Na^+ channel will permit lithium (but not K^+). It is of interest to note that occupation of Na^+ channels by Li is probable basis of lithium therapy of psychiatric disorders. Since in the membranes, electric current is carried by the ions, the channels are actually the routes by which the currents flow across the membranes. In the nerve membrane, Na^+,K^+ and Ca^{2+} channels play major roles in the production of action and other potentials. The generation of potentials is made possible by ihe flow of temporally regulated ion-carried currents through the channels. Based on experimental evidence obtained from biochemical and electrophysiological experiments, several models of channel structure and functioning have been proposed[4,51,52,118,119].

More information appears to have accumlated about the Na^+ channel[104] than about other channels. This is mostly because this channel plays a primary role in the nerve excitability. Therefore, it is worthwhile to examine the possible molecular structure and mechanism of operation proposed for this channel. The channel can be thought of as a protein macromolecule (*Fig. 2*) forming a pore-like passageway through the lipid bilayer of the membrane[29,52]. The wall of the channel is thought to bear a fixed negative charge. At the outer surface (*i.e.*, towards the extracellular side), entrance to the channel works as a filter responsible for the cation selectivity of the channel. Existence of a negatively charged group in the Na^+ channel which may be the chemical basis of the selectivity filter, has been demonstrated[50a]. At the cytoplasmic side of the channel, there is thought to be located a gate formed of protein molecule[52,119]. With this gate is supposed to be associated the mechanism by which the channel opens and closes. The gate also carries a charged electric field sensor extending into the lipid phase. The channel gating is controlled by the membrane potential changes perceived by way of its charged sensor[51,115]. The shifting of charges in the sensor results in an alteration of the molecular conformation of the gate which constitutes the opening of the passage. With the opening of the gate, the selectivity filter or pore located at the outer surface begins to let the ions flow through[51]. This is also to be noted that the gates are voltage sensitive whereas the selectivity filter is voltage insensitive[139]. The shifting of charges in the sensor is measurable as gating currents that occur immediately prior to the inward movement of Na^+ ions at the beginning of excitation. The channel appears to have only two conductance states, the open and the shut[115] and thus channel may pass from one state to the other in an all or none fashion. This simplified scheme of the functional architecture of the channel constitutes only a working hypothesis and is a good basis for further investigations. The channel has rather specific pharmacological properties. For example, the entry of Na^+ ions is blocked by tetrodotoxin (applied from the outside only) whereas the iodate, pronase (only when injected into the cell) act on the gate preventing its inactivation. The number of channels present in the membrane is probably not very high[131]. The number of channels present in tne membrane can be estimated by determining the concentration of tetrodotoxin that blocks the Na^+ channels. Some of the channel properties may also differ according to their location. For instance Na^+ and K^+ channels in axonal membrane differ in some of their properties from those in subsynaptic membranes. Na^+ and K^+ channels in axonal membrane are voltage sensitive while those in subsynaptic membrane are chemically sensitive and lack electrical excitability. Since electrical activity of the excitable tissues is the reflection of the dynamic activity of its ion-sensitive channels, and the ion-selective channels are present in nonexcitable cells also, it is of great interest to determine differences between channels of excitable cells and those of nonexcitable cells[96].

The Resting (membrane) Potential

After this brief introduction of pump and channel constituents of the excitable membrane, it is appropriate to consider as to how excitable cells (and other cells also) generate a potential difference across their membranes and how in response to the stimuli the potential undergoes changes to produce propagatable signals. In most cases the extracellular medium contains some cations and anions in concentrations characteristically different from those in the intracellular medium. There also exists a charge imbalance at the two sides of the membrane alongwith a nonuniform distribution of individual ion species inside and outside the cell. For instance in a particular case, for 108,000 Na^+ ions outside there are only 10,000 ions inside, and for 3,000 K^+ ions outside there are 100,000 K^+ ions inside. The Cl^- ion distribution is roughly the reciprocal of the K^+ ion distribution. Besides this, intracellularly there is a large population of negatively charged protein anions which are absent in the extracellular medium.

The activity of Na^+, K^+-ATPase pump is responsible for extruding, and maintaining a higher concentration of, Na^+ions outside the cell. With the extrusion of Na^+ ions, K^+ ions are brought in and this besides preventing an increase in the concentration of positive ions extracellularly brings the positive charges inside to make up the deficit caused by the expulsion of positively charged Na^+ ions. Since there are high molecular weight protein anions intracellularly, a fairly high concentration of K^+ ions is built up inside the cell. To balance the electropositivity produced by Na^+ ions in the extracellular medium, a suitable concentration of Cl^- ions migrates to the outside. The negatively charged Cl^- ions can be easily spared to come to the outside because of the availability of sufficient negative charges in the form of protein anions intracellularly. The protein anions because of their inability to pass through the cell membrane cannot reach the extracellular compartment.

The intracellular accumulation of K^+ ions creates an electrochemical gradient of potassium operating from inside to outside. As resting membrane is somewhat more permeable to K^+ than to Na^+, some K^+ ions diffuse back to the extracellular medium. However, this movement or leakage of potassium becomes, however, limited by the strong attraction exerted by the intracellular protein anions. K^+ ions provide the necessary positive charge for the neutralisation of the negatively charged amino acids, proteins *etc.* inside the cell. An equilibrium is finally established and further outflow of K^+ ions no longer continues. The leakage of K^+ ions to the outside produces an excess of positive charges in the otherwise electroneutral extracellular medium. Obviously the intracellular medium becomes negatively charged because of the escape of the K^+ ions from the otherwise electroneutral intracellular compartment. This is to be pointed out that it is the presence of impermeable protein anions in the intracellular medium that may be considered to have caused the redistribution of diffusible (permeable) ions across the membrane in terms of the principle of Donnan Equilibrium. The situation is however not simple because of the operation of the Na^+ pump that extrudes the Na^+ ions actively one way.

It is clear from the above account that although a variety of charges comes to be differentially distributed on the either side af the membrane, it is the leakage of K^+ ions to the outside that brings the outside of the cell at a positive potential (charge) relative to the inside which comes to acquire a negative charge. Thus, the value of the potential difference across the membrane (membrane potential) is approximately given by the K^+ equilibrium potential in accordance with the Nernst Equation[63]:

$$E_K = \frac{RT}{F} \ln \frac{[K^+]o}{[K^+]i}$$

Where E_K is potassium equilibrium potential, R is the gas constant, T is the absolute temperature, F is the Faraday constant, ln is the natural logarithm, $[K^+]o$ and $[K^+]i$ are the concentrations of K^+ ions outside and inside respectively.

Converting natural logarithm to logarithm at the base 10

$$E_K = 2.3 \frac{RT}{F} \log \frac{[K^+]\,o}{[K^+]\,i} \text{ mv}$$

$$2.3 \frac{RT}{F} = 58 \text{ (at } 18^{\circ}C \ \frac{RT}{F} \text{ is } 25)$$

and substituting K^+ concentrations

$$E_K = 58 \,.\, \log \frac{20}{400} \text{ mv}$$

$= - 75$ mv

as they are in the squid axon the value of potential difference across the membrane is given minus sign

Although directly measured values of the resting membrane potential (EM) are close to Ek but are rarely equal. The deviation of EM from EK is due to the contribution of other ions to the magnitude of the membrane potential values. Permeability of the resting membrane to the Na^+ is likely to make some Na^+ ions leak into the cell in accordance with their eleclrochemical gradient. As already noted, the leaked K^+ ions are the major determinant of the Em values, and keeping in view that the membrane is more permeable to K^+ than to Na^+, only a small contribution is likely to be made by leaked Na^+ ions. The basis and mechanism of the selective permeability of the resting membrane to one ion (K^+) than to the other (Na^+) is not clear. The type of channels that are open in the resting membrane will be an important factor besides the size of the ions themselves. Most of the involved cations in their hydrated form are smaller than Na^+. Consideration can also be given to the occurrence of voltage-independent leakage channels[20] as distinguished from voltage-dependent channels preferring one ion or the other. The presence of potassium-preferring voltage-independent leakage cation channels seem to be essential for the establishment of the resting potential at the nodes of Ranvier[20]. The concentration ratio of Cl^{+} ions across the membrane is about the reverse of the K^+ concentration ratio[63]. For example in case of the squid axon the situation is as follows:

$$\frac{[Cl^-]i}{[Cl^-]_0} = \frac{40}{560} = \frac{1}{16}$$

$$\frac{[K^+]_0}{[K^+]i} = \frac{400}{20} = \frac{20}{1}$$

Thus the potential difference produced by the Cl^- ions is about the same as by the K^+ ions. The Cl^- usually will not significantly contribute to the value of the membrane potential. In summary, Em can be considered somewhat equal to EK and deviations of the former from the latter are on account of the leakage of small number of Na^+ ions into the cell. To account for the contributions of various ions Goldman (1943)[37] devised a more elaborate equation:

$$Em = \frac{RT}{F} \ln \frac{P_K\,[K^+]_0 + P_{Na}\,[Na^+]_0 + P_{cl}\,[Cl^-]i}{P_K\,[K^+]i + P_{Na}\,[Na^+]i + P_{cl}\,[Cl^-]_0}$$

where P_K, P_{Na} and P_{cl} represent relative permeabilities of the respective ions. The relative contribution of an ion to the membrane potential will be proportional to its membrane permeability. Thus, greater the permeability of an ion, the larger will be its contribution to the membrane potential. K^+ permeability of the resting neuronal membrane is about 25 times greater than that of Na^+, and about 7 times than that of Cl^-. The above description concerning the generation of membrane potential is based on the data from squid giant axon which behaves as a Nernst potassium electrode. Electrogenic behaviour of the sodium pump has already been referred to. Goldman equation does not take into account the possible contribution from Na^+ pump. To account for the role that Na^+,K^+-pump may play in the generation of membrane potential, an equation has been given[128] :

$$Em = \frac{RT}{F} \ln \frac{\Upsilon K_0 + bNa_0}{\Upsilon K_i + bNa_i}$$

Where Υ is the coupling ratio *i.e.*. number of Na^+ ions extruded to K^+ ion brought in, and b is the ratio of Na permeability to that of K^+ *i.e.*, $\frac{pNa}{pK}$.

Apart from Na^+ and K^+ and Cl^-, one more cation that merits consideration with regard to the control of membrane potential is Ca^{2+}. Membrane potential as established by the differential distribution of various monovalent cations and anions exists in the presence of an stabilizing effect of Ca^{2+} on the membrane potential and excitability. Ca^{2+} binding to the membrane and its displacement from it appear to play some determinant role in the control of the membrane potential. Ca^{2+} ion concentration in the external medium normally is to be maintained within a given range. Significant departures may result in the destabilization of the membrane resulting in increases or decreases of the membrane potential[131].

Capacitance and resistance are the two physical properties of the membrane also which play some role in the membrane potential. These physical properties constiute passsive properties as distinguished from the active properties which refer to those involved in the current flow (ion conductances) across the membrane and the phenomena resulting from them (action potential etc). Obviously the membrane is an electrophysiological sytem and its passive aswell as active properties contribute to the electrophysiological phenomena manifested by it. The membrane, a high resistance lipoid-protein structure, behaves as a capacitor as it is like an insulator (barrier) placed between the two conductors, the intracellular and the extacellular media. The capacitive properties make it easier for the membrane to store (separate) charges across it. In the event of a current flowing through the membrane, the change in the membrane potential is not offset but is only slowed down. Current flowing through the membrane can take two pathways, one through its capacity and the other through its resistance, resulting respectively in capacitive and resistive currents. Current flow through the membranes can, to a certain extent, be described by the Ohm's Law. The membrane resistance influences the current-conducting ability (conductance) of the membrane. Since in nerve cells changes of potential across the membrane as well as propagation (conduction) of potentials over distances are central for the physiological functioning of the nervous system, a proper appreciation of passive properties together with the active ones is necessary. Propagation of the action potential largely involves active properties of the membrane because not only in its generation but also its conduction involves ion fluxes. Synaptic potentials, and generator potentials on the other hand are conducted from their site of origin without making use of active properties, although their production is based on ion fluxes. Thus, conduction of such potentials is subject to the passive properties of the membrane. The two parameters of the membrane, time constant and space constant, are determined by its passive properties. Time constant indicates the time course (rate) of change of the membrane potential when it moves from one level to the other, whereas the space constant refers to the rate of decay of voltage change with distance. When, for example, a synaptic potential is to propagate from the site of its origin to the trigger zone in the postsynatic neurone, a neurone with long time constant (length constant) will permit a more effective conduction to the trigger zone with minimal decrement[63]. Actual mathematical measurememts of these constants have been made in nerve cells and their relationship with resistance and capacitance has also been determined in several cases. For details reference can be made to Kandel (1976)[63]. Further attention will be given to these properties in a later section of this paper which deals with the integration of information.

The Action Potential

The existence of a membrane potential across the nerve cell membrane is indicative of that the membrane has considerable energy which upon stimulation can be released making a resting nerve cell membrane active. Incoming stimuli which may be in various forms such as neurotransmitter molecules released from the neurones, electric currents from the other chemical or physical stimuli from within or outside the organism, or stimuli applied by the experimenter, can interact with the polarized neuronal membrane at one or the other of its regions. The interaction with the excitable membrane ultimately results in a sequence of events that alter the membrane potential producing a transient wave of activity usually referred to as nerve impulse. The impulse has demonstrable and measurable electrical manifestations which constitute the action potential[114] that can propagate itself over long distances and can be transferred to other neurones mediated by special mechanisms.

It is usual in the laboratory to use electrical pulses as stimuli to study the mechanism (s) underlying the genesis of the nerve impulse. Imposition of such a stimulus (brief pulse of the electric current) to the axon membrane extracellularly, elicit a set of events in the macromolecular and multi-ionic mass of the membrane. When extracellular electrodes with a threshold current switched on are applied on to the surface of nerve fibre (axon), a current from the anode to the cathode would flow both through the thin film of the fluid over the outside of the fibre and the interior of the fibre crossing the membrane inward at the anode and outward at the cathode. Since current first flows through the membrane capacitance, the change in the membrane potential to be produced by the stimulus will begin to appear after some delay with the discharge of the capacitor. Charges delivered by the anode on to the surface of the membrane hyperpolarize it at this electrode; at the cathode, however, a depolarion takes place. This should be clearly understood that these initial potential changes are largely due to addition or subtraction of charges by the electrodes and it is the depolarization taking place at the cathode that is more relevant to the onset of excitation. With about 8 mv decrease (depolarization) in the membrane potential at the cathode, there begins some increase of Na^+ conductance (Na^+ influx) of the membrane which reduces the membrane potential futher. This conductance-related change in the membrane potential progresses with inceasing Na^+ influx contributing about 7 mv decrease to the potential. The first 8 mv change is called electrotonic potential change while the second 7 mv change is referred to as the local response. The former is due to changes in the passive properties (capacitance, resistance) of the membrane, and the latter to the active property (small ionic flow). At about 15 mv total depolarization (electrotonic+local), the Na^+ conductance is suddenly activated at a very fast rate as membrane permeability and conductance begin to increase enormously. With increased conductance, membrane potential falls further which in turn further increases permeability.

Relationship Between the resting Memberane Potential, Depolarization and Activability of the Na^+ Chemical

Changes in transmembrane potential gate the Nat+ channel. Activation of the channel is actually dependent on the level of membrane potential. At the resting membrane potential level, the Na^+ channel can be in a state of inactivation. A 15–20 mv decrease in membrane potential from the resting level activates the Na^+ channel to open permitting inflow of Na^+ ions. These channels become inactivated again at a potential 20–30 mv more positive (from 0 mv) than the resting membrane potential. Further, at this level of membrane potential no Na^+ current can be elicited as the Na^+ system is fully inactivated.

The two terms, conductance and permeability are quite often encountered in a description of the generation of action potential. A comment to clarify the distinction between the two will be appropriate here. Membrane electrical conductance ,of an ion reflects the ease with which a current is carried by ions across the membrane in response to the electrical gradients. It is the reciprocal of resistance and is the ratio of the flowing current to the potential difference which acts as a driving force:

$$g_{Na} = \frac{I_{Na}}{E_m - E_{Na}}$$

Where g_{Na} is Na^+ ion conductance, I_{Na} is net flow of Na current, E_m is the membrane potential and E_{Na} is the sodium equilibrium potential.

A distinction can be made between the electrical conducatance and permeability. Permeability indicates the ease with which a substance (uncharged) can pass through under the driving force generated by the concentration gradient and is defined by the diffusion equation which takes into account the concentration difference, electrical gradient and the thickness of the membrane involved. Permeability has the units of velocity. Since in neuroelectric events the diffusing substances are charged (ions), the definition of permeability becomes complicated as the velocity of diffusion depends on potential. The terms, conductance and permeability, are so often also used interchangeably, as in neurophysiological phenomena (*e.g.* excitation), because in such cases the two changes parallel each other. Mathematically permeability is given by the following equation:

$$P_{Na} = \frac{\mu \beta RT}{aF}$$

Where μ is the mobility of the ion, β, the partition coefficient a is the thickness of the membrane, P_{Na} is the permeability.

The velocity of ion flow is proportional to permeability but in a complicated way is related to the concentration difference and the membrane potential. The Goldman equation for membrane potential takes into account most factors.

It has been described that during interaction of stimulus pulse and the axon, the initial response of the neuronal membrane consists of a depolarization contributed by electrotonus and some Na^+ ion inflow. This initial response (depolarization) of the membrane to a level where it can trigger, in an all or none manner, a propagatable action potential can be referred to as stimulation. Obviously the changes take some time to occur indicating thereby that the stimulus currrent must flow through the membrane for some time before it can produce an action potential. Thus, duration of the stimulus pulse together with its strength (voltage) characterize the strength of the threshold stimulus. It also follows that the important molecular event that results following the application of stimulus and the initial depolarization enough for the threshold to be reached, is the activation of the Na^+ channels. Gating of the Na^+ channels leads to a rapid inrush of Na^+ ions driven by the concentration difference potential gradient. Na^+ ion invasion of the cell interior makes it transiently electropositive, whereas the corresponding region at the outside of the cell membrane becomes electronegative. Thus, the membrane potential level is shifted to a new value i.e., from–90 mv value to about +120 mv. A graphic plot of this change (potential change as a function of time) represents the first phase, the spike (upstroke/rising phase), of the action potential or potential of action. In terms of molecular and physicochemical changes it is indicative of opening of Na^+ channels, flow of Na^+ current and reversal of membrane electrical polarity. The upstroke lasts for about 0.5 msec and at its peak value Na^+ channels become inactivated (closing of channels); consequently the Na^+ current rapidly ceases. Another event that takes place at this time-point is the gating of K^+ channels. Opening of K^+ channels[58] leads to the flowing of an outward K^+ current which drives positive charges out of the cell under the driving force provided by the concentration gradient. A graphic plot of this phase represents the falling phase or the repolarization phase of the action potential reflecting increased K^+ permeability coupled with decreased Na^+ permeability of the membrane. The duration of the falling phase is longer than that of the rising phase. The outward K^+ current is in the direction of bringing the membrane potential level back to the resting level but sometimes for a while increase in K^+ current persists so that the membrane potential becomes somewhat more negative than the resting level value. This more negative component is referred to as the negative after potential. Alongwith the outward flow of K^+ ions, Na^+, K^+-ATPase pump is also transporting Na^+ ions outwardly. Na^+–pump–driven extrusion of positive ions also helps repolarization, although the Na^+ pump otherwise does not play a direct role in the production of action potential. Sometimes the negative after potential event is followed by a still another transient state of the membrane potential called as the positive after potential during which the membrane potential is less negative (more positive) than the resting level value. This will be mostly due to a greater than normal accumulation of K^+ for a while. With the termination of this event the membrane potential becomes re-estabished at the resting level value thus restoring the initial electrical polarity of the membrane. The various possible phases of the action potential are diagrammed in *Fig. 3.* The last two phases are not always represented and the time course and the amplitude of the spike can vary in different cases. Although generally there is a fast initial phase (spike), but during the second phase the return of potential to the resting level may be at widely differing rates. The action potential in mammalian cardiac muscle, for example, can have a duration as long as 200 or more milliseconds, with spike level being attained in less than 2 msec but the return to the resting level taking several hundred milliseconds.

During the action potential, the axon membrane has a refractoriness, that is, its excitability is lowered. The refractoriness (responsiveness) however does not have the same degree in each of the several phases of the action potential. During the rising phase of the potential, the membrane is absolutely refractory (not responsive) as it cannot be excited at all by any stimulus being applied during this period. During most part of the negative portion of the falling phase and in the following negative after potential, the membrane is in a state of relative refractoriness as it can be excited by a stimulus of the strength higher than that of the normal threshold. During the positive after potential even a subthreshold stimulus can excite the membrane as it is in a state of increased excitability because it is already depolarized to a certain extent. The cause of the altered responsiveness seems to lie in the degree of activatability of the Na+ conductance at different levels of depolarization in the various phases of the action potential. At the depolarization level that prevail during upstroke, the Na+ channel system is inactivatable and therefore the responsiveness of the membrane is abolished. However, during the negative part of the falling phase the Na+ channels are

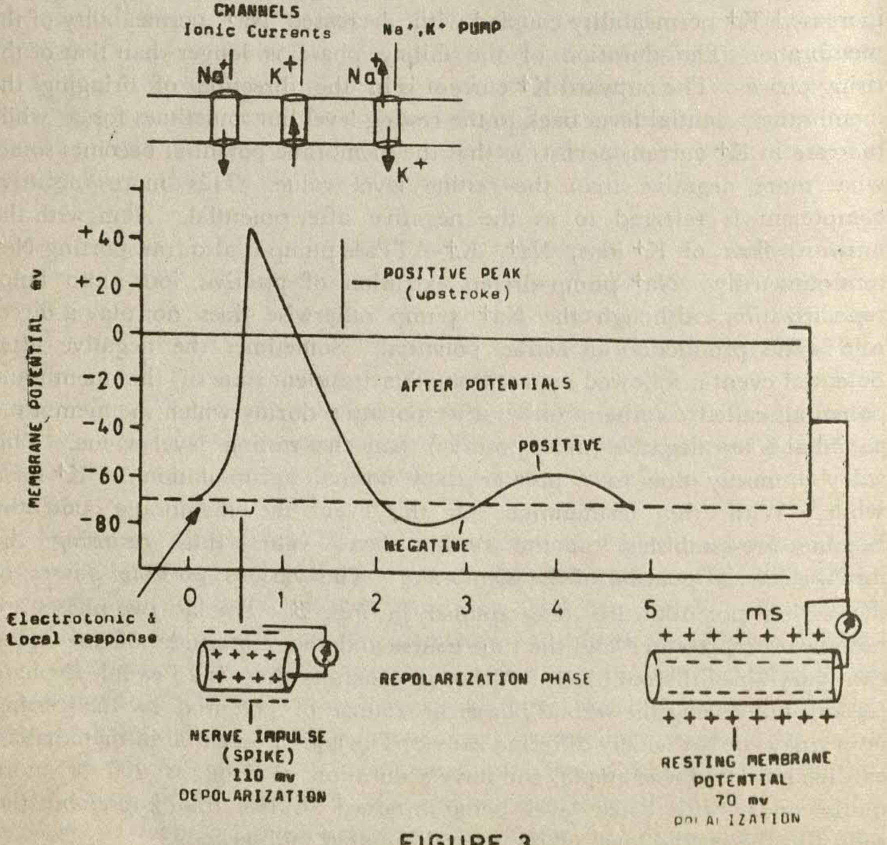

FIGURE 3

Fig. 3. Schematic representation of the phases of the action potential and the concomitant events in the membrane.

relatively activatable and thus the membrane begins to acquire responsiveness. The duration of the action potential and the duration of the refractoriness of the membrane are thus closely related to each other. Refractoriness limits the rate at which a nerve fibre can conduct action potentials. The duration of action potential will obviously put a limit on the interval of re-excitation. The property is of physiological significance, for example, in the case of cardiac muscles the prolonged refractoriness protects the musculature from too fast rexcitation.

Concerning the activatability of the channels in the axonal membrane, there are a few other points that can be considered. It has been mentioned that the channel has an electric field sensor. What is this sensor? How does it sense the electric field (transmembrane potential change) ? What changes does the sensor undergo to assume an open configuration *i.e.*, gating? These questions cannot, at present, be precisely and satisfactorily answered. Nevertheless these are important questions for which the answers will be particularly rewarding. At one time it was thought that it was actually the Ca^{2+} ions that comprised a sensor of the channel, and it could be believed that Ca^{2+} kept the Na^+ channels blocked (closed) and the transmembrane electric field change displaced Ca^{2+} charges to open the channels[31a] . The views according to which Ca^{2+} was modelled as sensor (or channel gate) have become untenable[51,52,139] and there appears to be no direct involvement of Ca^{2+} ions or other particular monovalent ions in sensing-gating process[51]. The energy required to produce gating currents is believed to come from the electric work done by the membrane electric field on some membrane components that is the voltage sensors[52]. These components can be regarded similar to the 'gating particles' postulated by Hodgkin and Huxley (1952)[55,56]. Ca^{2+} ions, however, have several modulatory roles on channels[52,131]. Membrane surface has negatively chargedg roups because of ionized carboxylic acid, phosphate sites etc.[51,115a] , to which monovalent as well as divalent cations would be attracted as counterions. Ca^{2+} is an important counterion to surface charges[51] and so has an important role in the stabilization of the membrane. Ca_2^+ modification of membrane charges may have some modulatory role on the channel molecules.

Involvment of Ion Other than Na^+ in the Generation of Spike

Generation of the rising phase of the action potential (*i.e.*, the inward current) is generally mostly regarded as a reflection of Na^+ inrush. There are, however, several observations indicating contributions from other ions to the initial inward current. Even in squid axon it has been found that alongwith the inward Na^+ current there is a minute inward Ca^{2+} current, which is further divisible into an early Na^+ conductance-paralleling, tetrodotoxin-sensitive component and a later external $Ca^2\pm$ dependent, tetrodotoxin and tetraethylammonium-insensitive but cobalt-sensitive component (for references see Triggle and Triggle (1976).[131] Besides squid, Ca^{2+} component of Na^+ action potential has been observed in several mollusc neurones.[68] In some other cases such as cardiac muscle cells, the Ca^{2+} current follows Na^+ current in action potential generation[99]. Further, evidence has shown that this Ca^{2+} influx utilizes a channel distinct from Na^+ and K^+ channels. The physiological significance of joint Na^+ and Ca^{2+} current in neuronal activity remains to be precisely understood. However, some observations are worth noting in this connection. In *Helix*, K^+ conductance of the action potential is turned on by Ca^{2+} influx during the rising phase of the action potential (Na^+ current)[87]. $Ca^2\pm$activated K^+ channels appear to be of wider occurence[93],[121] indicating thereby that the Ca^{2+} influx through gated channels associated with action potential may be of considerable physiological importance. Sometimes potential-activated K^+ channels can coexist with $Ca^2\pm$ activated K^+ channels. Action-potential-associated Ca^{2+} influx (current) is frequently found where intracellular Ca^{2+} concentation changes trigger intracellular events, for example, the neurotransmitter release[94],[126]. Ca^{2+} channels thus become more important at certain sites although they are otherwise widely distributed [32], [84a], [131]. Non neural cells such as pancreatic β-cells in which glucose induces a membrane depolarization leading to insulin release possess Ca^{2+}-activated K^+ channels [22] [23]. In these cells decrease in K^+ permeability mediates membrane depolarization. Of still further interest are cases where the cell body action potential has Ca^{2+} component whereas its axon utilizes only Na^+ for spike generation (i.e., the axon uses a mechanism different than the cell body in the same neurone)[62], [13].

Pharmacological Distinction of Channels

It has been already discussed that during action potential generation, Na^+ and K^+ channel gatings are temporally distinct and are subject to pharmacological modification in each one of them independently. A number of chemical antagonists are known which block selectively one or the other channal. Tetrodotoxin blocks entry of Na^+ into early Na^+ channels but spares K^+ current. Further, tetrodotoxin acts only on the outer membrane surface as it does not alter Na^+ channel inactivation mechanism nor it affects kinetics of Na^+ conductance increase or gating currents. These pharmacological observations show that distinct channel sites participate in various components of the channel function. K^+ channel current is sensitive to tetraethylammonium which reduces voltage-dependent K^+ conductance or of substitute ions through the K^+ channel. Further, both tetrodotoxin and tetraethylammonium are channel specific antagonists but not ion-specific antagonists. Ca^{2+} channels are antagonized by cobalt[23]. There are numerous data available concerning the pharmacology of channels[84a],[131] and such experimental approaches are of central importance to the understanding of channel physiology.

Examples of exitability Phenomena in Plant Kingdom

It will be appropriate to mention a few examples here from the plants which exhibit excitability phenomena somewhat similar to those of neurones. An interesting example is *Mimosa pudica*[103]. Long duration (several seconds) conducted action potentials, similar in form and amplitude (50-100 mv) to those of axon, that can travel along the petiole have been observed in *Mimosa pudica.* Absolute refractory period lasts for about 1 minute and is followed by a relative refractory period. Cells supporting the action potential appear to be located in the phloem and parenchymatous perixylem and possess resting potentials of -161 mv and-154 mv respectively.[103] This is of interest to note that electrical manifestations can be produced as responses to mechanical stimuli, temperature variations, light etc. *Chara, Nittela* are other examples in which extensive studies on electrical activity have been carried out [53, 67, 68]. In *Nitella axilliformis* the action potential is composed of an early inward current (Cl^-efflux) and a delayed outward current (K^+ efflux) besides some leakage current due to Na^+ and has a duration of 2-3 seconds. Electrical stimulation, in an all or none manner, can elicit the action potentials and the concerned cells have a resting membarne potential of about-110 mv. The membrane potential generation involves the activity of H± translocating ATPase electrogenic pump in plant cell membranes.[109a]

Propagation of the Action Potential

The action potential once generated is capable of self propagation. The mechanism by which it is accompolished is rather simple. The minute region of the axon supporting the transisent physiological wave of activity, the action potential, or the nerve impulse can be referred to as the excited region which is contiguous with the yet unexcited (to be excited) region of the axon. The excited region stimulates the adjacent unexcited area resulting in the generation of an action potential in the latter. The new potential represents the propagation of the nerve impulse from the previous point to the next. Note that the new action potential results from the necessary events (ion fluxes etc.) in the membrane portion that was as yet unexcited, the stimulus for which comes from the excited area. Since the excited region carries a negative charge at the surface and unexcited region a positive charge (and reverse inside the fibre), a local current between the unexcited and excited regions will begin to flow. The Na^+ ions diffusing inward in the action potential bearing region act as a current source for the electrotonic spread of the potential through the yet unexcited membrane site. The degree of the amplitude of the inward Na^+ current during the production of action potential determines its ability to propagate itself to the next region. There has to be sufficient current to flow away to the adjacent region in the interior of the fibre to be able to depolarize the membrane. If this current is not sufficient, the potential will become unconducted. In the case of synaptic potentials for example the amplitude of this potential is low and so, they are unable to conduct themselves by active machanisms. The action potential is conducted without decrement.

The current returning from the interior of the fibre and outwardly flowing up the membrane in the unexcited region depolarizes the membrane electrotonically and initiates generation of the action potential in the same manner as the externally applied stimulus pulse did in the previous region. That is, the initial depolarization is electrotonic and when it has reached a threshold level Na^+ conductances increases resulting in the fast inward Na^+ current producing the action potential spike and subsquently other components of the impulse. Meanwhile, repolarization is accompolished in the previous excited region. It is obvious that membrane currents flow at right angles to the direction of the movement of the impulse. This is in contrast to the electric current flow along the metalic wires. In the nerve fibre when an action potential has advanced from one site to the other, the former site could generate an action botential pecause of a membrane current from the newly excited region flowing back into the former site. The action potential, if produced, could cause repetitive excitation (reexcitation). This, however, in case of the conducted action potential is prevented by the high potassium conductance in the former site during its repolarization. In the cases where potassium conductance is not high enough repetitive excitation is likely.

Conduction Valocity of the Action Potential

Several factors are known to influence the conduction velocity of the action potential. These are potential-and time-dependences of the ion currents, membrane resistance, membrane capacitance, the fibre diameter, and other factors influencing the electrotonic spread of the current. Spatial advance of the action potential will depend on its amplitude because the amplitude determines the quantity of the current that can be available to be driven into the adjacent unexcited region. Thus, factors reducing the amplitude would tend to lower the velocity of conduction. Electrotonic spread is influenced by the membrane resistance and capacitance; the advance of the action potential would be significantly limited by these two properties as the initial events are electrotonic. Since capacitance and resistance of the unit area of the membrane are likely to be the same in most cases, the electrotonic spread will become related to the fibre diameter. The membrane area is proportional to the diameter of the fibre and the longitudinal resistance in the fibre also decreases with its diameter. The conduction velocity increases about as the square root of the diameter of the fibre[102]. Spike duration also seems to vary with the fibre diameter. At lower fibre diameter, the spike duration increases; the rising and falling phases of the impulse separately also show increases[90-92,102]. The spike duration thus seem to vary inversely with the fibre diameter. The velocity of propagation of the nerve impulse in the myelinated and unmyelinated nerve fibres differ considerably. For example 3m/sec is the velocity in a mammalian unmyelinated fibre, but a myelinated axon can conduct at the rates of 30 to 100m/sec. This is because in the myelinated fibres, the conduction is saltatory (leaping) i.e., the impulse skipping the myelin-covered portion of the fibre jumps from one node to the next. In the process, actually the current flows from one active node to the next (resting) node without appreciably affecting the axon segment covered by the insulating myelin sheath (internodal portion). The resting node is excited by the current coming from the preceding node. Active ionic currents occur only at the nodes (which are not covered by the myelin sheath) and so the conduction becomes speedier and economic. Voltage-dedendent Na^+ channels are present almost exclusively in the nodal axolemma, the internodal axolemma appears to be devoid of them. Although there is a resting potential and sometimes also there are voltage-dependent K^+ channels in the internodal membrane[20], the physiological significance of these is largely unclear. Frog myelinated nerves are known to possess both Na^+ and K^+ channels at their nodes, but mammalian peripheral nodes [20] and also central myelinated fiber[69] nodes lack K^+ channels. Repolarization in such cases is achieved through rapid Na^+ inactivation and leakage currents. It appears that K^+ channels exist in a masked state as disruption of myeline causes them to reappear.[69]

Other Theories of Excitation Procees

The mechanism of excitation described above is often termed as sodium theory and in general outlines the ionic basis of excitability [55,56,65]. This theory is most popular and the equations derived explain most of the experimental data[1]. However, other interpretations for the machanism of nerve excitability also exist[15,35,38,59,78,85,87,123,136]. It appears now rather necessary that instead of laying a rather exclusive emphasis on the electrical phenomena (ion currents, channel functioning, potentials), adequate consideration must be given to physicochemical changes in terms of thermodynamic parameters, ion selectivities, phase and conformational changes [59,11] long range spatial interactions and functional membrane units[87], and also to the intraneuronal compartment[2] for explaining the mechanism of excitability and neuronal function. Complexity of the action potential and excitability phenomena require a greater multidisciplinary approach for developing a rather comprehensive understanding of the subject.

Physical Changes

It is appropriate to consider some physical changes that occur in the axonal membrane during the excitatory process. Action potential production is accompanied by mechanical changes such as swelling in the fibre which are probably brought about by profound alterations in the membrane macromolecules at the axon surface[60, 125, 129]. These changes have been reported in squid, crab and possibly also in frog nerves. Studies of the optical effects of excitation[59] have shown that membrane properties such as birefringence and light scattering (indicating thickness and refractive index), fluorescence, polarization undergo changes during action potential production[65]. Since the total number of molecules involved in excitatory process in the axon is extremely small (for example in the squid axon about 0.002% support action potential), the physical changes occurring during the action potential are of a small magnitude and their molecular nature remain mostly unclear[65]. It has also been observed that in squid axon (giant), the intracellur pressure undergoes a brief rise and then a fall concomitant with the action potential.[127]

There are a number of other observations and data that cannot be easily explained in terms of the Na^+ theory of excitation process. A brief discussion of these is considered sufficient here. Drastic alterations in the ionic composition of the extracllular and intracellular media including the reversal of Na^+ ion gradients and also the complete absence of Na^+ in the extracellular medium often support the production of near normal action potentials [12, 59a 121, 122, 136]. Such observations were thought to indicate that the widely held view that Na^+ influx and K^+ outflux are the main basis of nerve impulse genesis, might not explain the excitatory process and gave support to the development of alternative interpretations of excitatory phnenomena and to other models of excitability. Impressive is the extensive work of Tasaki and coworkers [136] and their data make it possible to explain the excitatory process by mechanisms different than the Na^+ theory. According to this work, the excitation process is considered to be the result of the reaction of membrane macromolecules to the electrolytic ions in the extracellular and intracellular media. The reaction or better the response of the macromolecules respresents the assumption by the membrane of a rather stable excited state by the membrane. The interdiffusion of ions (ionic currents observed during the production of action potential is to be understood as a consequence of the establishment of a transtent stable excitatory state. The resting (unexcited) state of the membrane is also thought to be a resting stable state made possible by the occupation of negatively charged sites in the membrane particularly on its outer side by divalent cations particularly Ca^{2+}. The excitable membrane is thought to be capable of exhibiting two chemically stable potential states-one resting and the other active. The transition from the resting to the active state involves alterations in the configuration of the membrane macrolecules, which change their configuration because the stimulation cause changes in the intramembrane ionic environment as a result of a co-operative cation exchange by the molecules[136]. For these interpretations the membrane is to beregarded as consisting of an outer layer in which polyacids (ionized polyacid, phosphate groups) predominate and an inner layer which largely has polypeptide moiety. The outer layer, thus, can have negative sites to which divalent cations, mostly Ca^{2+} may be predominantly attrracted [51, 136]. That there are fixed negative charges at membrane surfane is widely accepted.[15] Replacement of Ca^{2+} by monovalent cations derived from the intracellular stores by electrical stimulation significantly changes the intramembrane ionic environment.

The difference between the Na$^+$ theory and the two stable state hypothesis of Tasaki seems to be that whether changes responsible for the production of action potential occur in one rather uniform molecular complex involving not necessarily any one ionic species or in discrete molecular separate entities (channels) independently of each other using distinctly one ion species in one channel[59],[124]. Recently, however, some observations of Tasaki group could be explained[59]a in terms of the ion channel theory. Other physical model of nerve excitability where greater emphasis is placed on the spatial molecular interactions during action potential generation, has been proposed[11],[78]. In this model, properties as are expected in equilibrium and dissipative structures of nonequiliburium systems[36] are considered to be of central importance. Some experimental data which show Na$^+$ channel to be a rather more elaborate macromolecular membrane complex containing multiple allosterically interacting components[19] appear to support such views. Morphological structures inside the nerve membrane that indicate the involvement of even cytoplasmic structures in the excitatory process have also been reported[108]. A rather recent study[6] reveals that some protein modifying agents when applied to the membrane intracellularly can produce conduction block in crab nerves, whereas no such effects appear upon their application from inside. These observations besides reemphasizing a distinction between the molecular organization of the outside and that of the cytoplasmic side of the membrane[51], [136] points out to the location of the stimulation sensitive material in the inner side of the membrane and this will be in accord with the models of excitability channels where electrical field sensor has been postulated to be present on the inner side of the membrane.

Does the active state of the excitable membrane involve only physical quantities : currents, potentials, conductance etc., and interaction of membrane protiens with electical field with no large molecular transformations.[110] as are supposed in Hodgkin-Huxley theory, or besides these somewhat greater molecular transformations (like phosphorylations etc.) are involved? A debate continues and several models of excitability based on greater role for intramembrane protiens in the genesis and maintenance of the excited state have been proposed [85],[87],[110]. It would, thus, appear that there is a lot more that is still to be understood about the molecular basis of the excitation process, and future work will make possible a synthesis of divergent views concerning the basis of excitation process.

Transmission of Action Potential Across the Synapse

The conducted action potential upon reaching the axon terminal meets a discontinuous passageway 'the synapse' which necessitates use of conduction mechanisms different than those involved in propagation along the continuous axonal fibre. At the synapse the two membranes – one belonging to the presynaptic structure (usually the axon terminal) and the other to the postsynaptic structure (usually the dendritic or soma membrane) are separated by a gap of some nanometers and have specific morphological and functional relationship with each other. These junctional gaps are functional contacts through which interneuronal communication and transfer of signals is possible. One more important feature of the synapses is that passage of signals across them is unidirectional i. e., from the presynaptic structures to the postsynaptic ones. Examples of synapses permitting bidirectional conduction are also there but are limited. The synapses can be established between the axon terminal and dendrite (axodendritic), axon terminal and cell soma membrane (axosomatic), axon terminal and axon terminal (axoaxonic), dendrites and dendrites (dendrodendritic) etc. Axodendritic synapses are rather more frequent and in these, axon terminals are the source of input to the dendrites of the postsynaptic neurone. The dendrites can provide, for example in cat motoneurone, as much as 80% of the receptor surface area for the synaptic inputs[5]. The simplest contact patterns are those in which only one axon makes synapses with one postsynaptic neurone. More often however, the axon terminals from many different neurones can establish synapses on the dendrites and cell body of the postsynaptic neurone which in turn may synapse with several others[5]. In a complex cluster of neurones such as the cerebral cortex, the cerebellar cortex, basal ganglia, hippocampus etc., in the brain tissue, the number of synaptic contacts will not only be numerous but also the pattern of interconnection by which they are established will also be extremely complex (Figs 1 and 4).

Based on the fundamental nature of the mechanism involved in signal transmission, two types of synapses can be distinguished, the electrical and the chemical synapses.

Electrical Synapses

Some synaptic junctions are characterized as electrical because the currents produced by the action potential in the presynaptic terminal can directly spread (flow) into the postsynaptic membrane to alter its conductance. The physical gap between the presynaptic and postsynaptic membranes in an electrical synapse is about 2 nanometers and is bridged by intercellular cytoplasmic channels (thus some continuity of the cytoplasm between the two cells) through which ionic currents can be efficiently passed[123]. The flow of currents through these channels is electrotonic and thus is decremental. The channels constitute rather low resistance junctional pathways, and allow even somewhat larger dye molecules such as Lucifer yellow to pass through. Electrical synapses can be rectifying allowing passage of current undirectionally, and also non-rectifying allowing bidirectional passage of current across them. It has been recently shown that in crayfish electrical rectifying synapse, property to let the dye pass

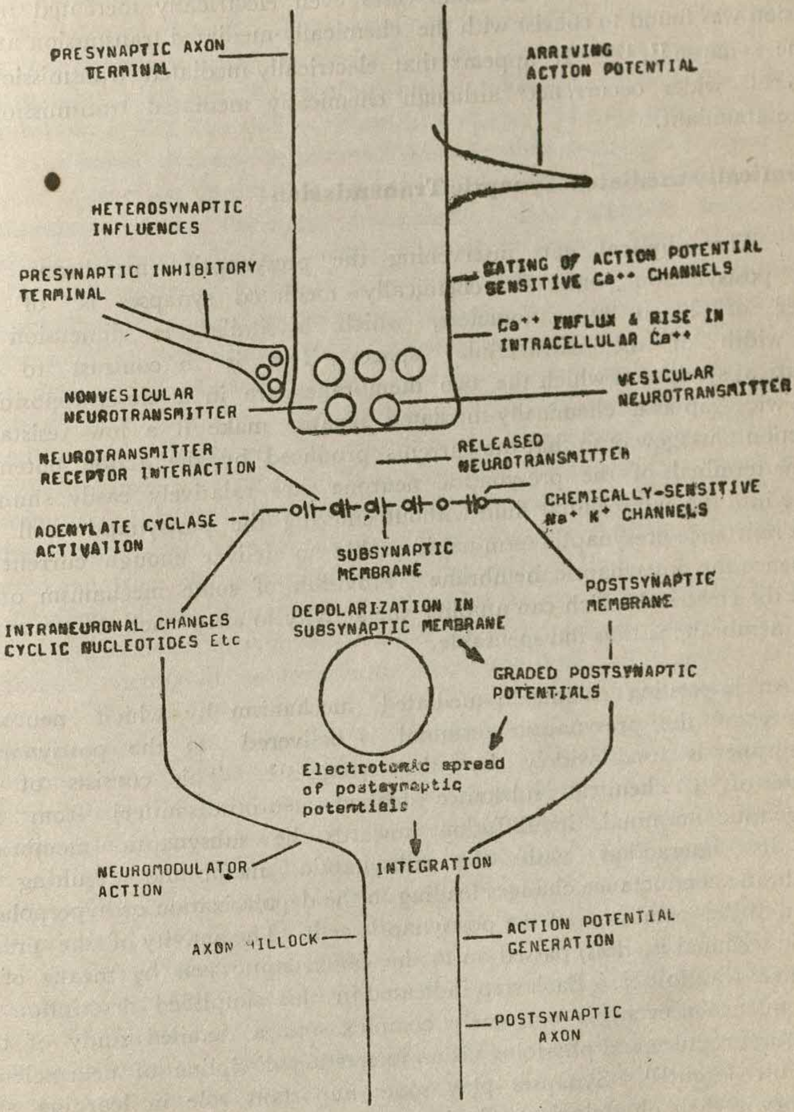
PRESYNAPTIC AXON TERMINAL
ARRIVING ACTION POTENTIAL
HETEROSYNAPTIC INFLUENCES
GATING OF ACTION POTENTIAL SENSITIVE Ca++ CHANNELS
PRESYNAPTIC INHIBITORY TERMINAL
Ca++ INFLUX & RISE IN INTRACELLULAR Ca++
VESICULAR NEUROTRANSMITTER
NONVESICULAR NEUROTRANSMITTER
NEUROTRANSMITTER RECEPTOR INTERACTION
RELEASED NEUROTRANSMITTER
CHEMICALLY-SENSITIVE Na+ K+ CHANNELS
ADENYLATE CYCLASE -- ACTIVATION
SUBSYNAPTIC MEMBRANE
POSTSYNAPTIC MEMBRANE
DEPOLARIZATION IN SUBSYNAPTIC MEMBRANE
INTRANEURONAL CHANGES CYCLIC NUCLEOTIDES Etc
GRADED POSTSYNAPTIC POTENTIALS
Electrotonic spread of postsynaptic potentials
NEUROMODULATOR ACTION
INTEGRATION
ACTION POTENTIAL GENERATION
AXON HILLOCK
POSTSYNAPTIC AXON

FIGURE 4

Fig. 4. Outline of the various events that may occur at synapses during transmission of signal across them.

through the synapse was distinct from the ionic conductance as the synapse was found to be permeable to the dye even in the absence of low ionic conductance[76]. The concentration of intraterminal Ca^{2+} (free) affects the efficacy of the electrical synapses[7]. Excitatory as well as inhibitory electrical synapses are known[33],[34]. In some cases even electrically mediated transmission was found to coexist with the chemically-mediated transmssion at the same synapse [77], [130]. It appears that electrically-mediated transmission is also of wider occurrence[8] although chemically mediated transmission is more abundant.

Chemically-mediated Synaptic Transmission

The physical gap intervening the presynaptic membrane and the postsynaptic one in chemically - mediated synapses is of the order of 20 to 30 nanometers, which is similar in dimension to the width of the extracellular space. This is in contrast to the electrical synapse at which the two membranes are in a closer apposition. The wide gap at a chemically-mediated synapse make it a low resistance junction passageway so that the currents produced by the action potential in the terminals of the presynaptic neurone are ralatively easily shunted away into the extracellular fluid without entering the postsynaptic cell. A high resistance presynaptic terminal is unable to deliver enough current to influence the postsynaptic membrane. Provision of some mechanism other than the electrical which can amplify the activity so as to reach the postsynaptic membrane is thus indispensable.

An interesting chemically-mediated mechanism by which neuronal activity of the presynaptic terminal is delivered to the postsynaptic membrane is used widely by the synapses[73]. This consists of a release of a chemical substance (called neurotransmitter) from the presynaptic terminal, its diffusion towards the subsynaptic membrane and its interaction with the subsynaptic membrane resulting in membrane conductance changes leading to the depolarization or hyperpolarization of the membrane of the postsynaptic cell. The activity of the presynaptic terminal is, thus, passed on to the postsynaptic cell by means of a chemical transmitter. Each step indicated in this simplified description of the sequence of events is extremely complex and a detailed study of the synaptic structure and physiology is an interesting discipline of neuroscience in its own right[131]. Synapses play some important role in learning and memory as their physiological effectivness or efficacy registers changes when they are frequently used. This implies that learning (uptake of information) leads to the production of some lasting changes in the synapses and the so altered synapses may constitute one important aspect of the physical form in which retrievable information can be stored'[102a]. Synapses are the sites of action of the drugs that can modify the functioning of the brain.

Presynaptic Terminal

A presynaptic terminal actually represents a terminal end part of one of the terminal branches of the axon and is usually referred to as a synaptic knob establishing contact with the postsynaptic neuronal membrane. The terminal is devoid of myelin. The axonal membrane of the presynaptic terminal has a greater density of Ca^{2+} channels. Interior of the knob contains characteristic structures called synaptic vesicles which accumulate and store the chemical substances that function as neurotransmitters. The synaptic vesicles are membrane bound dynamic structures. The chemical nature of a synapse or even the transmitter nature of a neurone[94]a is known by the neurotransmitter it synthesizes and uses for signal transmission. For example, designations noradrenergic terminal, serotonergic terminal, cholinergic terminal, gamma-aminobutyric acidergic terminal refer respectively to the synapses using noradrenaline, serotonin (5-hydroxytryptamine), acetylcholine, gamma-aminobutyric acid as neurotransmitters. A particular neurone synthesizes and stores one type of neurotransmitter. However, exceptions to this generalization have been found[35a]. The presence of the neurotransmitter is not limited to within the vesicile, but extravesicular transmitter pool within the synaptic terminal also occurs. The process of the transmitter release from the terminal is thought to involve mobilization of vesicles under the influence of the action potential, their fusion with the membrane of the terminal and eventual exocytosis of the transmitter into the synaptic cleft.

Invasion of the presynaptic terminal by the action potential is the first event in the sequence of events that evoke neurotransmitter release. Altered membrane potential of the presynaptic membrane leads to the gating of Ca^{++} channels. The resulting influx of Ca^{2+} ions increases their concentration in the terminal. The increased Ca^{2+} concentration is a link between the action potential and synaptic vesicle exocytosis (or release of transmitter). The magnitude of the intraterminal concentration of free Ca^{+} is the key factor in determining the amount of neurotransmitter that will be liberated from the presynaptic terminal[3,94]. The magnitude of the calcium influx and thus of the concomitant rise of intraterminal Ca^{2+} is dependent on the amplitude and duration of the presynaptic terminal action potential. A depolarization of the order of 25 mv is sufficient to raise the Ca^{2+} in the terminal to a level that can initiate neurotransmitter release at most synapses[63]. Further depolarization will augment the release. As the presynaptic terminal concentration of Ca^{2+} is correlated with the quantity of neurotransmitter that a terminal can release, changes in the amplitude of the presynaptic terminal action potential will also have a correlation with the amount of the neurotransmitter released. Factors that can influence the amplitude of the action potential at the presynaptic terminal would thus influence the release of the neurotransmitter. One such factor is the membrane potential of the presynaptic terminal itself. In some cases it has been found that small maintained depolarization of the presynaptic terminal membrane inactivates Ca^{2+} conductance whereas the reverse is true in the case of hyperpolarization. Synaptic effectiveness (capacity to release the neurotransmitter) can thus be modified by changes in the membrane potential of the presynaptic terminal. Release of the neurotransmitter from a given axon terminal is decreased if its membrane potential is somewhat reduced just when the action potential arrives (presynaptic inhibition). Similarly release can be increased if the presynaptic terminal is slightly hyperpolarized when the action potential arrives at it (presynaptic facilitation). A type of axo-axonal synapses has been found where axons of other neurones synapse directly on the presynaptic terminals of the neurones as is the case in the primary afferent sensory axon terminals of the dorsal horn of the mammalian spinal cord. These axoaxonal synapses[66] are chemical synapses and exhibit the usual features: synaptic vesicles, appropriate width of the synaptic cleft and the thickening of the subsynaptic membrane. Such synapses constitute the histological substrate of the presynaptic inhibition. A presynaptic inhibitory terminal induces depolarization of the membrane postsynaptic to it, *i.e.*, primary afferent axon terminal which itself is presynaptic to the spinal cord neurone. The release of the transmittar from the axon terminal on to the postsynaptic spinal cord neurone can be reduced by the activity of the presynaptic inhibitory synapse. The depolarization of the axon terminal resulting from the activity of the presynaptic inhibitory synapse decreases the amplitude of the action potential spike at the axon terminal. Besides presynaptie inhibitory terminal[140], presynaptic terminals causing increase (facilitation) in the amount of the neurotransmitter released are also known, for example in *Aplysia*[17],[18]. The existence of the presynaptic inhibition and facilitation clearly illustrate the influence of the membrane potential status of the axon terminal on the amount of the neurotransmitter release that is evoked by the action potential. These cases also illustrate how the efficacy at a given synapse can be influenced by the activity in the neighbouring synaptic pathways (heterosynaptic effects[88]).

It is not clearly understood as to how intra-axonal free Ca^{2+} mobilizes and or activates the synaptic vesicles to release their transmitter across the presynaptic membrane. It has been postulated that there exists a storage pool and a readily releasable pool of the neurotransmitter in the presynaptic terminal. The readily releasable pool refers to the neurotransmitter contained in the vesicles that are already very close to or in contact with, the axon terminal membrane where release sites in the membrane are located. The storage pool refers to the neurotransmitter contained in the vesicles that are somewhat away from the terminal membrane and are required to be moved to the release sites to get recruited in the releasable pool. Some observations tend to indicate that the intraterminal Ca^{2+} (rising) accumulates near the terminal membrane and probably stimulates the release sites causing fusion to them of the synaptic vesicles[81], [126]. The vesicles fused with the membrane are thought to open up and release their contents by exocytosis into the synaptic cleft[39,50]. The role of Ca^{2+} appear to be somewhat specific as accumulation of Mg^{2+} antagonizes the Ca^{2+} effect. Further, the synaptic vesicles are believed to exocytose their contents (neurotransmitter) in a quantal manner, i.e., each vesicle is assumed to contain a fixed macromolecular packet (one quantum) of neurotransmitter molecules and when it effects release by exocytosis all of its content is exocytosed, and from a terminal there is a simultaneous release of a large number of quanta i.e., synchronous exocytosis of a number of vesicles[50]. However, doubts have been expressed on the quantal nature of the release process[84].

The description given above conforms to the widely held view that the transmitter that is found in the synaptic cleft comes from the synaptic vesicles. However, there is also considerable evidence that can make one think otherwise. In a recent paper, entitled 'Nonvesicular Release', Tauc (1982)[126] has shown that there are strong reasons to think that evoked release of neurotransmitter could be nonvesicular rather than vesicular. In this regard a few points merit mention. Major support for the vesicular release hypothesis is the presence af the synaptic vesicles itself. It is really enigmatic as to why electron microscopy has not been able to satisfactorily and unequivocally demonstrate exocytosis[50]. The examples of cases where transmitter release occurs in vivo in the absence of the synaptic vesicles or similar structures show that existence of vesicles itself does not unequivocally indicate the fact of vesicular release[50]. In the cholinergic neuromuscular junction it is known that there is some spontaneous (unevoked) release of the neurotransmitter from the presynaptic terminals and this release is nonquantal and in part Ca^{2+} dependent and sometimes even can be evoked by stimulation. It is of interest to consider how spontaneously released acetylcholine crosses the presynaptic terminal membrane. A study of the release of the neurotransmitters from the cortical synaptosomes presents evidence[48] that carrier-mediated transport of the neurotransmitter across the membrane is a distinct probability. That transmitter can be extruded by the cells at regions where vesicles or similar structures do not exist shows that ability to release transmitter is not limited to the synaptic terminal. There are several other points of interest given by Tauc[126]. Vesicles can serve functions other than transmitter release better: buffering of extra calcium, its (Ca^{2+}) extrusion etc. Thus, the debate whether the neurotransmitter release is vesicular or nonvesicular is alive.

In the foregoing account it has been described that it is the action potential spike at the presynaptic terminal that triggers the process of transmitter release. Besides such synapses, there exists a class of synapses which is activated by a graded depolarization of the presynaptic membrane rather than by an action potential spike[42]. In such cases, there is a threshold depolarization of the presynaptic membrane which causes the release of the neurotransmitter that acts on the postsynaptic membrane. Synapses not using action potentials, for example, occur in retinae photoreceptor. cells, horizontal cells, bipolar cells[14, 42, 142]; in these cases the presynaptic terminal releases neurotransmitter as a function of the membrane potential. Further, amacrine cells of vertebrate retina[137] and some neurones of lobster stomatogastric ganglion[79] use both mechanisms: graded membrane depolarization-evoked and action potential-evoked release of neurotransmitter at their synapses.

Phospholipids and Synaptic Function

It has been generally known that stimulus-induced alteration of membrane potential gates tne ionic channels and subsequent ionic flux such as Ca^{2+} influx at the presynaptic terminals initiates or triggers a sequence of events. Evidence has also accumulated to show that alongwith the depolarization-triggered Ca^{2+} influx in synapses, there is a specific hydrolysis of membane phosphoionositides to diacylglycerol and inositol triphosphate[9],[43],[47],[89], the former probably acts by stimulating phosphorylation and the latter by mobilizing intracellular Ca^{2+} to produce intracellular responses associated with synaptic events. The phospholipid effect is thought to constitute a fundamental transducing mehanism[9] mediating stimulation and neurotansmitter effects at synapses. Some observations make it possible to suggest that the phospholipid effect may even be associated with the opening of Ca^{2+} gates[83]. A wide variety of extracellular signals (hormones, electrical stimuli etc.) cause phospholipid inositol turnover. The responses of the cells to Ca^{2+} concentration changes appear to be distinct from those produced by diacylglycerol and it seems that the two sets of events act synergistically in bringing about the physiological response[89]. Such studies are beginning to unfold the role of membrane phospholipids in synaptosomal excitability in which proteins hitherto have been enjoying an almost exclusive importance. Since it has already been known that pump as well as channel molecules require phospholipids for their activity, the changes occurring in phospholipids during excitation process must have some importance. Ca^{2+} no doubt has a central importance. Its ability to stimulate neurotransmitter release from vesicles is mediated by several intervening steps. The presence of calmodulin-like protein associated with synaptic vesicles has also been shown[26]. This portein is a receptor protein for Ca^{2+} and many of the latter's effects appear to be mediated by it including stimulation of protein kinases. The recent evidence shows that stimulation-triggered perturbation of phospholipids also appeared to be associated not only with Ca^{2+} mobilization but also with other events such as activation of protein kinases, guanylcyclases etc.[9].

Action of Released Neurotransmitter on the Post Synaptic Membrane

Before examining the mechanism of action of presynaptically released neurotransmitters on the postsynaptic membrane it will be appropriate to briefly review the chemical nature of neurotransmitters. Since neurotransmitters are the agents of neuronal communication and changes in their metabolism can have a direct influence on behaviour, these have always been, and continue to be, of intense research interest in neurosciences. About 50 chemical substances, so far, have been indentified as putative transmitters[10]. According to the chemical nature, the neurotransmitters can be grouped under several categories:

Amino acids—Glycine, glutamic acid, gamma-aminobutyric acid etc., have been found to act as neurotransmitters at several types of synaptic junctions. Glycine and gamma-aminobutyric acid generally serve as inhibitory neurotransmitters whereas glutamic acid is an excitatory transmitter.

Amneis—There are 4 monoamines which act as neurotransmitters: epinephrine (adrenalin), noradrenalin (norepinephrin), dopamine and 5-hydroxytryptamine (serotonin). The first three together are called catecholamines, they are formed from the amio acid tyrosine. 5–hydroxytryptamine is synthesized from the amino acid tryptophan.[93a]

Acetylecholine—is another important neurortransmitter. It is synthesized from choline and acetic acid (acetate).

Neuroactive peptides–A number of peptides are known which act as neurotransmitters in strict sense such as substance P which is made up of 11 amino acids. Transmitter neuropeptides act fast and over a short distance altering the excitability of the neurones. Some neuropeptides function as neuromodulators as they alter the response of a neurone to a distinct chemial input[45], [109,138]. Endorphins are other examples which modulate the conduction and processing of the pain in the brain. Some other neuroactive peptides function as neurohormones; they act at a distance from the site of release. Hypothalamic releasing hormones are the examples.

Presynaptic endings of a particular neurone contain one type of neurotransmitter. However, this view that a neurone produces and releases only one type of neurotransmitter has been recently challenged. In an identified neurone of the lobster nervous system, dopamine and serotonin have been shown to coexist[25a]. The case is of particular importance because the two classical neurotransmitters were found in the same neurone. Several other reports had shown the existence of a classical neurotransmitter together with a neuroactive substance such as peptides in the same cell. Recent observations[10] seem to make it amply clear that more than one type of neurotransmitter can be stored in a presynaptic terminal[54a]. There are also instances of the neurones which can change their neurotransmitter status, for example in response to the environmental stimuli[10]. There are examples of neurones in which a classical transmitter and peptides have been found to coexist in the terminals[54a,116] although the physiological significance of coutilization of these is not clear.

Presynaptically released transmitter diffuses across the synapse and interacts with the specific receptors located in the postsynaptic membrae. Neurotransmitter-receptor interaction results in the membrane conductance changes due to alterations in the ionic permeabilities of the activated membrane. The postsynaptic membrane may either be depolarized or hyperpolarized i.e., the membrane potential is either brought closer to 0 mv or is shifted away from the resting level. Depolarization leads to excitatory postsynaptic potential, the transmitter involved being termed as an excitatory transmitter, whereas hyperpolarization results in an inhibitory postsynaptic potential and the transmitter involved is termed as inhibitory transmitter. Since the same neurotransmitter can be excitatory at some synapses and inhibitory at others, the nature of the postsynaptic response (inhibition or excitation) is actually determined by the nature of the receptors present in the postsynaptic membrane. With respect to acetylcholine, three types of postsynaptic cholinergic receptors are known. In the same way there are at least two types of adrenergic receptors. The postsynaptic receptors, appears to be closely associated with the relevant ionic channels. Thus if the transmitter--receptor-channel interaction changes the conductance (ionic current flow) in a hyperpolarizing direction, the postsynapic cell will be prevented from firing an action potential. However, if the transmitter-receptor-channel interaction alters the ionic conductance in a depolarizing direction, the postsynaptic cell membrane will be activated to fire an action potential. It should be noted that ionic channels in the postsynaptic membrane are chemically activated channels and not the voltage-activated ones which dominate the axonal membrane. Chemically activated nature of these channels refers to their responsiveness to the chemical action of the neurotransmitter plus receptor and in this property they are distinguishable from the membrane potential-gated channels of the axonal membrane. In some reports on *Aplysia* eurones voltage sensitivity of the postsynaptic chammes was observed[30]. Postsynaptic membrane channels can be named in accordance with the neurotransmitter receptor they are associated with. For exam-plecholinergic Na^+ ion channel refers to the Na^+ channel which is activated following interaction of acetylcholine and its postsynaptic membrane receptor. In the case of gamma-aminobutyric acid receptor, it is the Cl^- ion channel that is associated with the receptor [100]. Therefore, acetylcholine interaction with the receptor will result in the opening of Na^+ channel leading to depolarization whereas in the case of gamma-aminobutyric acid reccptor, Cl^- channel will open causing hyperpolarization. The ion conductance changes produced by transmitter-receptor interaction may vary. For example, in the production of the excitatory postsynaptic potential there may be a sudden increase of Na^+ conductance ; in many cases Na^+ conductance and K^+ conductance may simultaneously increase and not sequentially as in the case of action potential. In some cases, reduction of K^+ conductance may result in excitatory postsynaptic potential [63]. Inhibitory postsynaptic potential might be tne result of an increase in the membrane permeability to K^+, or to Cl^-. Sometimes inhibitory postsynaptic potential can also be produced by turning off Na^+ conductance [63]. This should be noted that in the case of postsynaptic potential the Na^+ conductance does not regeneratively increase because the subsynaptic membrane Na^+ channel is not voltage dependent. The Na^+, K^+ channels involved here appear to be pharmacologically different from those in the axon, as they are not sensitive to tetrodotoxin and tatraethylammonium respectively.

Integration and the Response of the postsynaptic Neurone

The description given above would mostly appear to be representative of a case where a single presynaptic terminal impinges upon the postsynaptic neurone. Usually many presynaptic terminals not only from one neurone but also from several others may make synaptic contacts with a single postsynaptic neurone[40]. In the case of the spinal cord motoneurone of cat, most input is received at the dendrites constituting over80% of the receptor suface area[5] in the The response of the postsynaptic neurone will depend upon the spatio-temporal pattern of the input[101]. The flow of the ionic currents across the postsynaptic membrane at several synapses may be represented by the following equation[5]:

$$Is = gs\,(t)\,[Vrev - V(t)]$$

Where Is is the net current flowing into the neurone, gs(t) is the conductance change resulting from ionic permeability changes, V (t) is the potential difference across the membrane at a time t, and Vrev is reversal potential i.e., changed potential resulting from the synaptic current.

The quantity Vrev−V (t) represents the synaptic driving potential. The synaptic current flow is proportional to the synaptic conductance change, and also to the driving potential. The synaptic current flow brings the postsynaptic membrane potential towards the reversal potential (Vrev) which is determined by ionic permeabilities of the synaptic membranc. For excitatory synapses the Vrev is close to zero mv i.e., from its resting level, the transmembrane potential will have been driven towards 0 mv (aqual to -70 mv depolarization). In an inhibitory synaptic event, the Vrev will be of a value away (more negative) from the resting level i.e.,-70 mv to -80 mv (hyperpolarization).

A single excitatory synaptic terminal will be able to produce only a very small change in the potential of the soma of the postsynaptic neurone. One quantum can produce a change of about 100 microvolts as in the case of the motoneurone soma of cat[5]. Thus, many such synaptic inputs must sum together to drive (depolarize) the resting membrane potential to the threshold level. Once the membrane potential of the cell body of the neurone exceeds threshold (reversal potential), the cell begins to fire the firing rate (frequency of the action potentials) of the postsynaptic neurone is roughly proportional to the magnitude of the total depolarization produced by all the individual synaptic events. The neurone soma thus functions like an integrator of many synaptic inputs. The site where the action potential is actually initiated in the postsynaptic neurone lies close to the initial portion of the axon (axon hillock), which probably corresponds to the region of the lowest threshold. It also follows that individual synaptic events from input sites at the dendrites or on the cell body are required to spread electrotonically across the cell membrane towards the axon hillock which actually does the addition (integration). Since the total input points on the soma of a postsynaptic neurone may include both excitatory and inhibitory inputs, the process of integration can involve addition of both types of inputs. Temporal as well as spatial aspects of the integration process are important and these are influenced by the time and space constants of the concerned neurone. If the time constant is sufficiently long, the synaptic potential produced by an event will rise slowly and will decay also still more slowly than the synaptic current. The potential change consequently will last long enough to be added to the consecutive potential changes resulting from the succesive synaptic inputs (temporal summation). The magnitude of the time constant is determined by the capacitance of the membrane involved. Spatial aspects of the integration are also of significance because the synaptic potentials are required to be propagated over some distance from the site of their origin. Thus, longer space constants favour the spreading of the potential with minimal decrement to the trigger zone. The spatial summation constitute the procecs by which the cell sumumate the spatially discrete synaptic inputs. Depolarization of the trigger zone(axon hillock)to a threshold level makes the cell to initate action potentials. Output at thc trigger zone is thus a balance of the excitatory and inhibitory inputs received by the neurone. Since mostly a large number of neurones are involved, the output of a group of neurones is similarly a balance of the inhibitions and excitations. An interesting example of simple level integeration is seen in *Paramecivm*. It is known that if the anterior end of this organism is stimulated, a depolarizing reeptor potential is generated with the production of a Ca^{2+} action potential and the animal begins to move backwards. But if the posterior end is stimulated, a hyperpolarization results and the animal moves forward with increased velocity. If the two ends are stimulated simultaneously, the direction the animal will adopt for movement will depend on the membrane potential determinded by the summation of depolarization and hyperpolarization. For this phenomenon *Paramecium* has been referred to as a swimming neurone[86a] and the process of integration occurring in it would appear to be analogous to that of neurones.

A few more points regarding the neurotransmitter receptor interaction need mention in connection with the response of the postsynaptic neurone.

The conducatance change produced in the postsynaptic neurone has been found to be associated with the activation of membrane adenyle cyclase[61] and the neurotransmitter receptors appear to control the activity of adenyle cyclase[111]. Adenyle cyclase activation results in the formation of cyclic nucleotides from ATP[135] which through the activation of particular protein kinases constiute intraneuronal processing of the information[2]. Synapses can control their function by alteration of the neurotransmitter release. They are thus plastic structures as they can modify their function. Recent studies indicate that synapses can be replaced, can increase or decrease in number when required, and sometimes even the dendritic arborizations can become elaborate with age[25]. Repetitive transmission of an event (signals) across the synapse can facilitate its response to the same signal in future. One of the important examples of such stimulus-evoked modification of the synapses is post-tetanic potentiation[75] which represents an excitatory postsynaptic potential having a rather long decay time and is indicative of a physiological change that represents the trace (or print) of the activity that has traversed through the junction. It has been generally hypothesized that physical memory record is somehow stored as modified physiological effectiveness or efficacy of the synapses[102a].

After the neurotransmitter has interacted with its receptors, termination of neurotransmiter activity is necessary to prevent its continued action at the synapse. The neurotransmitter action can be terminated by one or more of the following mechanisms: reuptake of the neurotransmitter into the presynaptic terminals, enzymatic breakdown (degradation of ihe neurotransmitter, rapid diffusion of the transmitter in the intercellular fluids[131]. Example of a synapse using gamma aminobutyric acid (GABA) sufficiently illustrates the points just mentioned. GABA is an important inhibitory neurotransmitter of the mammalian bɹain. It also occurs in the invertebrates. This amino acid neurotransmitter is of further interest because of its involvement in the epileptogenic disorders[72,95]. In GABA-ergic neurones, GABA is synthesized from glutamic acid which is enzymatically decarboxylated by glutamic acid decarboxylase. It is also to be noted that glutamic acid is not directly taken up by the neurones of the brain as it is unable to pass through the blood-brain barrier. It is, thus, glutamine that is first converted into glutamic acid which gives rise to GABA. The synthesis of GABA is known to take place exclusively in the nervous system. GABA thus formed is accumlated in synaptic vesicles.

Presynaptically released GABA interacts with its receptors located on the postsynaptic membrane where it activates Cl^- ion channels. Increased Cl^- conductance leads to membrane hyperpolarization. The mechanisms that contributes to the termination of GABA action are as follows. Some GABA molecules are removed by reuptaking by presynaptic terminals through Na^+-dependent high affinity (binding) membrane pump. The binding of GABA to its postsynaptic receptors is Na^+ independent. Inside the terminal, GABA is reaccumulated by the vesicles. Some of the free GABA can be broken down to succinic semialdehyde, the reaction being catalysed by a mitochondrial enzyme GABA-transaminase. The succinic semialdehyde can be further converted to succinate (the reaction is catalysed by a dehyprogenase) which can be utilized by the Kreb's cycle. Extra GABA still left in the synaptic cleft is catabolized by neuroglial cell mitochondria forming succinic semialdehyde and then succinate which as already mentioned can be used by the Kreb's cycle. Glutamic acid decarboxylase is a rate limiting enzyme of the pathway. The glutamic acid decarboxylase-catalyzed reaction is practically irreversible whereas the other two steps are reversible. Measurements of the activity of glutamic acid decarboxylase of the synapses can provide information about the excitability level of the tissue[72]. The affinity of GABA to its postsynaptic facilitated by the drug benzodiazepine which seems to modulate operant function of GABA receptor-chloride channel complex[100] and this mode of action of the drug seems to be the basis of its therapeutic antianxiety effects. The membrane effect of benzodiazepine is receptor mediated. There seems to exist a natural endogenous protein which can modulate GABA action[45]. GABA release at some axo-axonic synapses is known to be involved in presynaptic inhibition.

The neurotransmitter norepinephrine is formed from the amino acid tyrosine through a multistep enzymatic pathway and accumulates in the synaptic vesicles. The termination of the postsynaptic action of norepinephrine is accompolished largely by presynaptic reuptake (Na±dependent high affinity pump) of the transmitter and its reaccumulation in the vesicles. Some free norepinephrine in the presynaptic terminal can be metabolized by the enzyme monoamine oxidase. Some of the free norepinephrine still remaining in the synaptic cleft is metabolized by catechol-o-methyltransferase after extraneuronal uptaqe; together with this some catabolization can be carried out by monoamine oxidase also. Termination of the action of serotouin (5–hydroxy–tryptamine) is also achieved by a high affinty reuptake and catabolism by monoamine oxidase.

Presynaptic Receptors

In some cases, receptors occur on the presynaptic terminals for the same neurotransmitters which the terminals release[74,86,97]. Interaction of these receptors with the released neurotransmitter can modulate the neurotransmitter release process by a negative feedback mechanism. In the case of central and peripheral noradrenergic terminals[97], it has been found that excess neurotransmitter present in the synapse can depress its further release by the stimulation of specific presynaptic receptors. In the case of dopaminergic terminals, it seems that the dopamine release is modulated by the action of dopamine on the presynaptic receptors but in this case release is depressed indirectly through an inhibitory action on tyrosine hydroxylase (decreasing transmitter synthesis). Mention can also be made of the coexistence of the functionally opposite receptors for the same neurotansmitters on the same postsynaptic neurone. For example, dopamine released from one neurone can produce both excitation and inhibition on the same postsynaptic neurone[12]$_0$.

The output (resultant activiiy) of a cluster of neurones (cell group or nucleus) is a balance of the interaction of excitation and inhibition generated by the inputs[59b] received by it through presynaptic activity. Changes in the magnitude of the excitatory and inhibitory influences can be reflected in the activity generated in the postsynaptic neurones of the group and thus in its output. That is, if excitatory influences exceed the normal and their effect is not dampened by a corresponding increase of the inhibitory signals, the output will be an overexcitation. Hyperexcitation can also result from a reduction of inhibitory influence without a corresponding decrease of excitatory influence. Similarly an excessive inhibition can result from the absence of excitatory influences or from strong inhibitory influence reaching the postsynaptic neurones. Thus, a group of neurones or a subsystem of neurones (as in the case of brain) can be so altered as to become hyperexcitable exhibiting an excessive burst of action potentials. Such a discharge of hyperactivity if passed down through a motor tract can land to the production of convulsions. Hyperactivity can itself lead to altered behaviour and sensation. Similary excessive inhibition would produce behavioural effects. Such changes are known to be associated with clinical conditions: epilepsy (hyberactivity), mania (hyperexcitation), depression (hypoactivity) etc. At cellular level, these disorders may result from a derangement of neurtoransmitter metabolism (synaptic events) and or from causes within the neurones[95,115b]. Epilepsy-like convulsive disorders have been found to occur naturally in some animals. Experimentally, a group of neurones can be made hyperexcitable to some extent exhibiting features similar to those occurring in natural clinical cases. Animal experimental models in which epileptogenic states can be induced chemically or by other stimuli help understand cellular and molecular mechanisms underlying clinical situations. How and why does activity in a particular group of nerve cells in brain result in a particular type of psychological event i.e., pleasure, sadness, aggression etc.? These are questions which will engage attention of researchers for all times to come. Synapse pharmacology is an area of considerable interest in this regard. It has become possible to alter behaviour through modification of synaptic functions by pharmacological means. Synthesis and accumulation of neurotransmitters by synaptic vesicles, release of neurotransmitters form the presynaptic terminals, action of neurotransmitters on the postynaptic receptors are the steps that can be individually interefered with pharmacologically. Modification of behaviour or restoration to near normal behaviour can be achieved by manipulating pharmacologically the neurotransmitter-linked synaptic events. Synapses in the laboratory can be isolated as synaptosomes through ultracentrifugation and physiologcal and pharmacological observations made on them reveal considerably about the in vivo functioning of synapses[71,72,131].

Since the submission of this paper to the Editor's office, a number of publications of interest have appeared. Although incorporation of all of them at this stage will not be possible, reference to a few of them is being given here. Some more reports have provided evidence for the coexistence of more than one classical neurotransmitters in the same neurone: GABA and serotonin in the neurones of the dorsal raphe nuclei of rat[145], GABA and histamine in the neurones of the magnocellular nucleus of posterior hypothalamus of rat[171], GABA and dopamine in hypothalamus of rat[160]a. GABA and dopamine in the neurones of the main olfactory bulb of rat[161]. Widespread colocalization of neuropeptides has been shown in the individual neurones of rat visual cortex[165].

There have been a number of reports on the role of Na^+, K^+-ATPase in neuronal activities: correlation between neuronal activity and the levels of Na^+, K^+-Atpase activity etc.[143,171], membrane potentials, release of neurotransmitters, electrogenicity[147], [155], [167], influence of sex hormones and involvement in reproductive functions[157,149]. It is of interest to note that brain Na^+,K^+-ATPase activity appears to be a useful indicator of neural activity for the study of neuroendocrine interactions[149]. An interesting paper on the maximum transport capacity of the Na^+K^+ pump has also appeared[148]. There is a review dealing with the influence of lipid environment on the transport proteins in cell membranes[152]. Some reports concerning the mechanism of action of lithium with nervous tissue are also worth mentioning since they appear to throw light on its antimanic and other actions in psychiatric disorders. Although "pathways" for the transport of lithium across the axonal membrane of squid have been demonstrated, lithium's ability to substitute for Na^+ in the generation of the action potential appear to be of no relevance in relation to its therapeutic effectiveness[154]. Reported data indicate that lithium can depress synaptic neurotransmission by inhibiting mechanisms involving receptors linked to phosphoinositide metabolism which comprise a major second messenger system mediating action of neurotransmitters and hormones[159,162].

There have been many reports on ion channels and their involvement in excitability and generation of action potentials. In mollusc neurones, it has been demonstrated that Ca-dependent K currents constitute a powerful spike repolarization mechanism in addition to voltage-gated K currents[156]. Inactivation of K^+ conductance by Ca^{2+} has been demonstrated in bull frog sympathetic neurones[174]. Role of blockade of K^+ channels in the generation of neural bursting has also been studied.[158]. A study by Belardetti et al[144] has looked into the mechanism associated with the opening of some types of K^+ channels. Influence of the blocking of K^+ channels on action potential characteristics was also studied by Targ and Kocsis[172]. They showed that pharmacological blockade of the channel with 4-aminopyridine lead to broadening of action potential as well as a variety of excitability changes.

Regional cooling of human sensory nerve increased the amplitude and surface area of the evoked sensory compound action potential and these changes were thought to be due to cold-induced slowing of Na^+ inactivation. A paper by stimers et al [170]a is of interest regarding gating currents in the Na^+ channel. A molecular genetics approach is noteworthy in the report of Noda et al[164] in which synthesis of functional sodium channels using messenger RNAs generated by transcription of the relevant cloned cDNAs encoding the rat brain sodium channel polypeptide has been achieved. The mRNAs were found to direct formation of functional sodium channels when injected into *Xenopus* oocytes.

As an example of non-nervous cells exhibiting electrical excitability, mammalian pituitary cells have been found to show spontaneous and evoked electrical potentials[158]. Presence of voltage-gated Na^+ and K^+ channels was shown in rat astrocytes, however there has been no evidence that they can generate action potential[146].

Concerning the specificity of neurotransmitter-receptor interaction in synapses involving the mediation by postsynaptic adenylate cyclase, the report of Deterre et al[151] is of interest. this is shown that mollusc (*Helix aspersa*) neurones contain distinct serotonin and dopamine sensitive adenylate cyclasses. Pharmacological and electrophysiological data have been given to illustrate distinctiveness of the two types of adenylate cyclases. Concerning the biochemical mechanisms underlying the development of synaptic plasticity associated with learning etc., a report by Routtenberg and Lovinger [168] is of interest. It has been found that long-term potentiation in intact hippocampus is associated with selective increase in the phosphorylation state of 47-kDa protein. This change in protein state may be related to the electrophysiologically-defined alteration in synaptic strength. That acetylcholine released from the presynaptic terminals results in a burst of channel openings rather than a single opening has been shown in rat superior cervical ganglion[150].

A review has been published on the theory of presynaptic chemical neurotransmission[163]. In a report by Sandoval et al[169], it has been shown that intraterminal increases in sodium concentration can stimulate transmitter release as sodium can act as a physiological releaser of calcium from intracellular stores. Existence of adrenergic presynaptic receptors has been shown in GABAergic terminals in the hippocampus[166]. These receptors are involved in the modulation of GABA release. Concerning physical changes that are likely to occur during synaptic neurotransmission, Tasaki et al[173] in the bullfrog sympathetic ganglia found rapid swelling of presynaptic terminals as well as of the postsynaptic neuron.

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9

BIOLOGY OF ASSOCIATIVE MICROORGANISMS DIGESTING CARBOHYDRATES AND NITROGENOUS MATERIALS

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Introduction

Cellulose is the most abundant of all naturally occuring organic compounds, probably comprising at least a third of all the vegetable matter on the earth. The biological degradation of cellulose has been of paramount importance in the activities of the living systems. As a general rule, plant products are cycled through animals before man can obtain the necessary nutritive proteins from his environment. During this active cycling process, the efficiency of conversion of the plant products to animal proteins is dependent upon the digestibility of cellulosic products by animals.

In the rumen, different species of bacteria and protozoa interect through symbiotic relationship and generate characteristic products such as cellulose, hemi-cellulose and starch by digesting plant polymers. Bacteria metabolize on these substrates to form acetate, propionate, butyrate, carbon dioxide and hydrogen. Methanogenic bacteria utilize CO_2 plus H_2 and from methane. Some secrete urease to cleave urea to form ammonia and carbon di-oxide, some also synthesize B-complex group of vitamins. The cell contents of plants are lipids, sugars, organic acids and several water soluble proteins, sugars, organic acids, starch, non-protein nitrogenous compounds and pectins.

Cellulose decomposition, a complex process mediated by a series of enzymes, is carried out by a wide variety of organisms. In soils, decomposition can be accomplished directly through the activities of fungi or aerobic and anaerobic bacteria capable of degrading cellulose to glucose and mixtures of acids. In invertebrates, indirect degradation is believed to be affected by the production of enzymes by microorganisms inhabiting alimentary tracts of the insects. The most interesting and well studied is the cellulose digestion by termites which subsist on a diet rich in cellulose, which may be in the form of living or dead wood, woody tissues of plants, humus and dung.

Though vertebrates including human beings do not have enzymes which can cleave β-configuration of 1-4 glycosidie linkages, yet the anaerobic microorganisms in the rumen help in the digestion of cellulose to liberate enormous amount of energy. The interest in the rumen system has arisen principally because most animals of agricultural importance are ruminants or near ruminants. The rumen microbial ecosystem is unique because similar organisms and reaction found in the gut of non-ruminants herbivores but they are also found in the intestines of omnivores such as man. Dietary proteins are hydrolysed by rumen microbes at favourable H-ion concentration (pH 6.5) ; soluble sugars available in rumen are readily utilized by microbes to use up ammonia. Ammonia is utilized for microbial protein synthesis. Energy sources such as starch, corn, molasses, cane molasses and beet molasses increase the rate of conversion of urea or other ammonium salts to microbial protein of higher biological value.

The main theme of this review is to compile the current information available on the associative microorganisms involved in various metabolic activities *viz.*, cellulose digestion, metabolism of proteins and other nitrogenous compounds including role of microbes in dinitrogen fixation both in termites, ruminating and non-ruminating biological system.

Associative Microorganisms

The dietary components of the ruminant feeds are mostly cellulose, hemicellulose, starch, xylans, proteinaceous compounds and vitamins. The microflora both cellulolytic and non-cellulolytic[104] inhabiting the digestive tracts actively take part in converting the dietary material to the comparatively consumable volatile fatty acids, namely, acetate, propionate, aldehydes, methane, carbon dioxide and hydrogen. Microorganisms involved for this specialized metabolism comprise of Protozoa, Actinomycetes, Fungi and Bacteria. Table 1 summarizes the list of organisms commonly occuring in the guts.

Table. 1. Microbes commonly inhabiting the guts

Cellulolytic Microorganisms

1. *Diplodinium* (Protozoa)

2. Phycomycetes (Fungi)

   Neocalcimastrix frontalis
   Sphaeromonas cummunis
   Piromonas communis

3. Bacteria :

   Bacteroides succinogens
   Streptococcus sp.
   Ruminococcus fiavifaciens
   Enterobacter sp.
   Ruminococcus albus
   Staphylococcus sp.
   Butyrivibrio fibrisolvens
   Bacilus cereus
   Flavobacterium sp.
   Arthrobacter sp.
   Alcaligens sp.
   Serretia sp.
   Bacteroides sp.

Non Cellulolytic Microorganisms

1. Holotrichs (Protozoa)

2. Entodiniomorphs (Protozoa)

3. Bacteria :

   Bacteroides ruminicola
   B. amylophilus
   Succinovibrio dextronosolvens
   Succinomonas amylolytica
   Treponema sp.
   Lachnospira multiperus
   Eubacterium ruminantium
   Megosphaera elsdenii
   Selenomonas ruminantium
   Veillonella alcalescens
   Streptococcus bovis
   Lactobacillus vitulinus

Strict anaerobic bacteria *viz.*, *Clostridium lochheadii Cl. longisporum*, *Cellobacterium cellulosolvens*[29,76] have been implicated as cellulose digesters. Polysaccharides of fibre, in general are difficult to hydrolyse, but xylan fermenting *Butyrivibrio flbrisiolvens*, *Bacteroides ruminocola*, *Enbacterium ruminantium* and pectin hydrolysing bacteria, *Lachnospira multiparus*[152] were also reported from the digestive tracts of several animals. There are also reports of glycerol fermenting microbe, *Anaerovibrio lypolytica*.

Prokaryotes responsible for extensive secondary fermentations of metabolites have also been reported from the rumens. *Selenomonas ruminantium* convert lactate or succinate to acetate or propionate. CO_2 and H_2 with the mediation of *Methanobacterium ruminantium*, metabolise to yield methane.

Matuszynska and Janota—Bassalik[97] have isolated an actinomycetes from rumen of sheep and proposed the name *Micromonospora ruminantium* which hydrolysed starch, carboxymethyl cellulose and natural cellulose. Mainly lactic, acetic, propionic and butyric acids were produced from the fermentation of cellulose.

Protozoan fauna include ciliates and flagellates. Ciliates are strict anaerobes and presumably they feed on the rumen bacteria for their protein and vitamins need and the ruminants digest the ciliate possibly for their nitrogen source[23,36,41,47]. Hungate[79] has also reported the ruminants as the planktons feeder. planktens come through the food herbage or plant material. To a large extent, termites are dependent on the symbiotic flagellate, bacteria, actinomycetes and fungi for digestion of polysaccharides. The hindgut of many termites is packed with cellulose digesting protozoa, bacteria such as spirochaetes and is considered the site of considerable microbial activity. It is estimated that 65–99 per cent of cellulose and hemicellulose undergo degradation while passing through the termite gut[49] and in wood feeding termites 54–93 per cent is digested[171].

In lower termites (Mastotermitidae, Kalotermitidae, Hodontotermitidae, Rhinotermitidae and Serritermitidae) the hindgut contains large unmber of anaerobic prokaryotes characterized for cellulose digesting properties. They are *Oxymonads*, *Trichomonads*, *Hypermastigotes*[71]. Yamin[176,178] isolated a number of cellulolytic species *in vitro* from termite hindgut of *Zootermopsis*, *Trichomitopsis termopsidis Trychonympha sphaerica*. Both the species required cellulose and could not be replaced by glucose or cellobiose.

An analysis of the gut flora *Odontotermes obesus* commonly encountered in dry and semi-arid areas in India has revealed the occurence of several fungl (Table 2). The body surface and the gut surface of the termite also harbor various types of fungi (Table 3). Gut of termites have also been implicated to maintain the nitrogen recycling bacteria. Termite mound soils have been critically analysed for nitrogen cycling bacteria. Data seem to reflect the presence of large number of bacteria assigned to play conspicuous role in the nitrogen metabolism (Table 4).

Common Cockroaches, *Eublaherus pasticus*, *Periplanata americana* are reported to harbor cellulose digesting bacteria.[43] Isolated bacteria were

Table. 2. Fungi isolated from the Termite Gut (*Odontotermes obesus*) growing in Semi-Arid areas in India

Fungi	Individual colonies/pe ml homogenised gut suspension
Cunninghamella echinulata	44
Penicillium sp.	120
Fusarium monoliformes	33
Aspergillus awamori	68
A. flavus	87
A. nidulans	79
A. clavatus	73
Rhizopus stolonifer	87

After Rajagopal, Rajlakshmi and Varma, 1981.

Table. 3. Associative Fungi of *Odontoteemes obesus*

External body surface :

- *Aspergillus clavatus*
- *A. Nidulans*
- *A. flavus*
- *Penicillus sp.*

On the Gut surface :

- *Aspergillus clavatus*
- *A. nidulans*
- *A. flavus*
- *Penicillum sp.*
- *Cunninghamella echinulata*

Termite infested wooden pole :

- *Paecilomyces fusisporus*
- *Alternaria alternater*
- *Penicillum sp.*
- *Cunninghamella enchinulata*
- *Cladosporium sp.* and
- *Rhizopus sp.*

after RajGopal and Varma, 1980. Current Science, 49 : 632-636.

Table. 4. Degradation of nitrogeneous compound Mean No. of bacteria in samples from mounds of *Macrotermes spp.* and adjacent soil samples (0.10 cm) from Rhodesia

Bacteria	No. /g	dry wt	Significant differences
	Mound	Soil 0.10 cm	
Nitrifiers : Nitrite-oxidizers	943±423	13±6	P = 0.05
Nitrifiers : NH_3 oxidizers	4808±858	938±305	P = 0.001
Ammonifiers (X10^{6})	37 .7±4	24.6±4	P = 0.05
Clostridium Sp (X10^{3})	197±55	474±146	N.S.
Beijerinckia sp.	156±46	393±121	N.S.
Denitrifiers : *Bacillus sp.* (X10^{3})	356±69	196±44	N.S.
Denitrifiers : *Pseudomonas sp.* (X10^{3})	159±54	4.6±1.3	P = 0.01

c.f. Meikeljohn, J. (1965)

Microbiological Studies on Larger Termite Mounds

Rhod. Zamb, Mal. J.Agric. Res. 3, 67-79.

capable of solubilising Carboxy methyl cellulose and natural cellulose (Table 5) *in vitro*. A number of bacteria and fungi solubilising cellulose *in vitro* have been isolated from gut of millipedes *Orthosporus ornats* and *Glomeris marginata*[5,154]. Several reports are available in literature for the occurence of cellulose solubilising microorganisms from small wood eating insects.[144,157]

The principal source of nitrogen is ammonia in rumen for the microbial growth. Ammonia comes from protein and urea in the degraded form. The proteolytic activity is conducted by protozoa, large and small bacteria. Epithelial cells of the rumen harbour large number of bacterial cells,[75] including species of *Bacternides*, *Seleomonas* and *Butyrivibrie*[1,17,58,63,64].

Table 5 Cellulolytic Microorgansims from Cockroach Gut

Bacteria	Cellulase activity	(ug of glucose/h/mg protein)
	CMC	Cellulose
Clostridium sp.	21.1	8.72
Eubacterium sp.	25.2	11.27
Serratia sp.	47.4	13 15
Citrohacter sp.	21.5	8.47
Klebsiella sp.	25.5	9.82

After Cruden and Markovertz.[43]

There are also report for the occurence of rumen bacteria responsible for sulfur degradation. [149]

A wide range of proteolytic bacteria exist in the rumen of several animals, the most commonly encountered proteolytic forms are species of *Selenomona*, *Butyrivibrio*, *Lachnospira* and *Peptostreptococcus*.[58] Enzyme peptidase are located externally (exopeptidase) and internally (endopeptidase).

Amino acids are formed due to proteolysis led by several protozoa, *Entodinium caudatum* [37,124,] *Eudiplodinium* (or *Metadinium*) *medium*. [114] Several other ciliates expected to be proteolytic judged by their incorporation of bacterial amino acids, are *Entodinium longinucleatum* [126], *Ophryoscolex spp*[96,167,] *Epidinium eucaudatum caudatum* [38,39,] *Endiplodinium maggii* [40] and *Isotricha sp*[65].

Lev [95] studied glutamine stimulated amino acids and peptide incorporation in *Bacteroids melaninogenicus*. *Bact. ruminocolo* synthesize isoleucine and *Streptococcus bovis* arginine. Enzymes responsible for amino acid production from ammonia have also been reported from rumen bacteria. [28] Urecolytic bacteria also occur in rumens. The forms responsible for urease activity are species of *Staphylococcus Micrococcus*, *Corynebacterium*, *Streptococcus* and *Propionibacterium*[341·05,162]

The biological degradation of cellulose in the soil has been of paramount importance in the activities of the living systems. In semi-arid soils, rates of decomposition of organie compound are limited by available water, nitrogen and carbon. Decomposition and nutrient cycling are, therefore, key processes affecting primary production of esential elements in semi-arid regions. Cellulose decomposition, a complex process mediated by a series of enzymes, is carried out by a wide variety of organisms. In soils, decomposition can be accomplished directly through the activities of fungi, or aerobic and anaerobic bacteria degrading cellulose to glucose and mixture of acids. Millipede species are detritivores, as they feed on soil, plant litter and other items found on the soil surfaces. The highest proportion of the plant detritus comes from the litter and dead roots but exudates and slaughed cells from growing plant roots and foliar leaching make a significant contribution. Soil fauna together with other animals and their excreta make a comparatively small annual contribution whilst microbial and biomass make significant contribution. Information indicates that active termite mound soil contain bacteria (Table 6) would produce C_1 (active upon crystalline cellulose), and Cx (active upon non-crystalline cellulose, and soluble derivatives and degradation products of cellulose) cellulases and β-glucosidases or cellobiases (ative upon cellobiose). Informations indicate that much of the food that these millipedes ingest has already some what degraded by free living microbial flora (bacteria and fungi) possessing ncessary C_1 and Cx cellulases. This would also be a form of millipede dependence on microbially produced enzymes. The association between millipede and termite mound soil bacteria may be mutualistic. We have recently [127] isolated six mesophilic and one thermophilic bacterial strains capable of digesting cellulose in aerobic conditions from termite mound soil located in semi-arid areas in northern India. Table 7 shows the cultural and physiological characterisitcs of two most efficient isolates solubilising cellulose. These strains belong to the member of coryneform group after comparing with the reference strain *Cellulomonas flavigens.*

Table 6. Occurance of bacteria from live termite mound soils located in semi-arid areas

Collection	Viable Colony counts/g soil	Cellulose solubilising bacteria/g soil
Winter 1982	5.3±3.2x10-7	4
Monsoon 1983	8.6±4,7x10-7	6
Winter 1983	3.8±2·9x10-7	2
Summar 1984	Few in number	None
Monsoon 1984	7.7±2.8x10-7	5

Collection of samples was randum form the wild virgin area of the Jawaharlal Nehru Univiesity Campus, New Delhi. Environment cultures were made on minimal basal medium cotaining 1.3 g/l agar. Glucose or cellulose or cellobiose was added as carbon energy source. Incubation was done upto 28 days at 35^{0}C. Sodium bicarbonate was added to buffer the incubation suspension. Data are mean of seven replicates.

Table 7 Discriptive chart of Cellulose Utilising organisms-strain DGRP$_1$ WRS$_1$

Characteristics	DORP$_1$	WRS$_1$	*Cellulomonas flavigen* (Reference strain)
Morphological Characteristics			
Form	Short rods, curved	Long rods, straight	Rods curved
Size	0.28x0, 5-0 9/µ	0.7-0.8x1. 4/µ	0.4 6.0.7-1 8/µ
Motility	Nonmotile	Motile	Nonmotile
Gram strain	Positive	Positive	Variable
Cultural Characteristics			
Nutrient agar (Difco)	grow feebly	grow feebly	Smooth, glistening opaque, yellow
Yeast extract agar (Difco)	Dark orange, small, circular colonies, raised opaque,	white, large cicular colonies, opaque raised	—
Brooth	Uniformly turbid	Uniformely turbid	Uniformely turbid
Optimal temp.	33-37^0	35-37^0	30-33
Biochemical characteristi			
Gelatin liquefaction	slow	fast	slow
Methyl red test	Negative	negative	—
Nitrate reduction	reduced to NO_2	reduced to NO_2	Reduced to NO_2
CM-Cellulose gel hydrolysis	positive	positive	positive
Voges-proskauer test	negative	negative	negative

Digestion of Carbohydrates

Type of Carbohydrates

Carbohydrates include sugars, starch, cellulose, gums and related substances. Though corbohydrates form the largest part of feed which is fed to livestok, yet very little sugar and glycogen is present in the animal tissues. Commonly consumed carbohydrates are:

1. *Mono saccharides*
   Pentoses ($C_5H_{10}O_5$) : arabinose, xylose, ribose
2. Hexoses ($C_6H_{12}O_6$) : glucose, fructose, galactose, mannose

2. *Disaccharides* ($C_{22}H_{22}O_{11}$) : Sucrose, maltase, loctose, cellobiose
3. *Trisaccharides* ($C_{18}H_{32}O_{16}$) : raffinose
4. *Polysaccharides*
   Pentosans ($C_5H_8O_4$)x : araban, xylan
   Hexosans ($C_6H_{10}O_5$)x : dextrine, starch, cellulose, glycogen, inulin

   Mixed polysaccharides : gums, mucilages
5. Other related compounds : Uronic acids, hemicelluloses, Polyuronides lignin.

Cellulose is one of the organic substances occuring in abundance in nature and is a great potential source of energy for ruminants, and some lower organisms like termites and some arthropods. Cellulose is an insoluble polysaccharide composed of as 10,000 β-(1–4) linked glucose residues although only monomeric glucose, the dimer cellobiose, and low number oligosaccharides are taken up by microorganisms. The cellulose chains are arranged in structures known as fibrials which contain highly ordered stable crystalline regions and more loosely bound amorphous zones. The proportion of crystalline to amorphous cellulose will affect microbial and enzyme penetration, and therefore, susceptible to hydrolysis. Though vertebrates do not have enzymes which can cleave β-configuration of 1-4 glycosidic linkages, yet the microorganisms present in the rumen, gut of termites help in the digestion of cellulose to liberate enormous amount of energy.

A partial list of the most abundant high molecular weight substrates is given in Table 8 and details of their nature and occurrence can be seen in a number of reviews.[30'44'53'113]

Hemicelluloses are plant cell wall heteropolymers composed of a mixture of hexose sugars, pentose sugars and uronic acids. A third polysaccharide, pectin, is a minor yet important constituent of plant tissues and has a highly variable structure based on a methylated polyuronide. Lignins are the most complex of the abundant plant derived substrates and are polymers of coniferyl, sinapyl and p–coumaryl alcohols joined by a variety of intermonomer linkages whilst the likely monomeric costituents of lignin (e g. vanillin, syringaldehyde, guaicylglycerol, coniferaldehyde) are absorbed and metabolized by a large number of microbial species.[33]

Thus cellulose, hemicellulose and pectin in combination with lignin form a physically and chemically integrated structure that presents a series of problems to microorganisms utilizing plant cells as growth substrates.

Table. 8. Major macromolecular substrates, their origin and structure

Macromolecular substrate (origin) 1	Structure 2	Molecule enteric cell[b] (known or suspected) 3
Cellulose (P, M)	β-(1-4)-D linked glucan	glucose, cellobiose
Hemicelluloses (P)	β-(1-4)-D linked xylan glucouronans galacturonans xyloglucan	xylose, xylobiose glucuronic acid, galacturonic acid.
Pectin (P, M)	Xyloglucan	Xylose
Starch (P, M)	α-(1-4) and (1-6) linked glucans	glucose, maltose
Lignin (P)	Polymers of P-hydroxycinnamyl alcohols.	Monolignols, di- & trilignols
Chitin (A, M)	β-(1-4)-linked N-acetyl glucosamine	N-acetyl glucosamine
Proteins and Peptides(A,M,P)	Polymers of amino acids	Amino acids, low number peptides
Lipids (A,M,P)	Triglycerides, Phospholipids	glycerols, fatty acids
Peptidoglycen (M)	Polymers of N-acetyl glucosamine and N-acetyl muramic acid with peptides	N-acetyl glucosamind. N-acetul muramic acid, amino acids and low number peptides
Microbial exopolysaccharides	Mannans, dextrans, levens, xanthans, pullulan alginate	Mono and disaccharides

a, origin ; P, plant ; A, animal ; M, microbial ;

b, differs for different microorganisms

c.f.[31]

Digestibility of Carbohydrates in Rumens

In rumens, hemicelluiose and pectins are well digested whereas lignin is completely indigestible. Lignin influences digestiblity only when it is in cell wall and this is why certain grasses with low lignin content but more cell-wall have digestibility less than legumes with twice the lignin content. It is because legumes have on an average much lower cell-wall content than grasses.[142,151] Lignin reduces digestibility of carbohydrates by hydrogen binding at critical sites which limit cellulose action. Cutin, a non-aromatic structure, is another component of cell-wall which prevents microbial digestion. Alkali treatment of straws improves palatability, nutritive value and even growth rate.[61] Better nutritive value seems to be due to dissociation of lignin from the cell-wall by alkali treatment which aids in adhesion of greater number of cellulolytic bacteria such as *Ruminococcus flavifaciens* and *Bacteroides succinogenes* to unlignified cell walls.[90] Latham *et al.*,[91] using electron microscopy, observed the adherence of *Ruminococcus flavifaciens* and *Bacteroides succinogenes* to sodium hydroxide.treated barley straw and the less lignified stem of the grass *Lolium termulentum*. Before alkali treatment, while both bacteria adhered to and digested the unlignified walls of the innermost parenchymal cells, there was very little or no adherence to other areas of the stem. After NaOH treatment there was much more geneneral adherence bacteria being associated with most type of cell-walls. Athough the bacteria adhered to cell walls shown by histochemical methods found to be of reduced lignin content, this did not necessarily result in polysaccharide degradation.

The cellulose decomposing bacteria isolated from sugar cane fields, *Cellulomonas*, hydrolysed and decomposed 90% sugarcane bagasse within 5 days when bagasse was treated properly with alkali and heat.[62] The pattern of cellulose and hemicellulose fermentation in rumen is shown in the Fig. 1. a, b.

In order to determine the chemical nature of alkali attack upon straws, the main effect is probably that alkali removes phenolic acid residues acting as barriers to microbial degradation of the polysaccharides. The straw digestibility can be improved to a certain extent but there finally remains a lignified structure which cannot be further opened up by the low temperature alkali treatment. Evans,[52] has given more detailed review of chemical and physical aspects of alkali treatment. Alkali treatment removes some of the lignin and allows more extensive bacterial colonization and degradation.

Digestion of Cellulose Depends on the Diet of Ruminants

When cereal grains are included in the diet of ruminants, fiber or cellulose digestibilty of the roughage component is generally reduced. The addition of 20 to 30% glucose to a 2:2:1 timothy hay-corn-cotton-seed meal ration was found to decrease fiber digestion by about 25 *per cent* in both calves and sheep. This reduction in fiber digestion could be caused by a depletion of noncarbohydrate nutrients by the saccharoclastic bacteria, by an excess production of acid, or by an inhibition of cellulose by soluble sugars. Using mixed cultures of rumen bacteria *in vitro* and nylon bag techniques

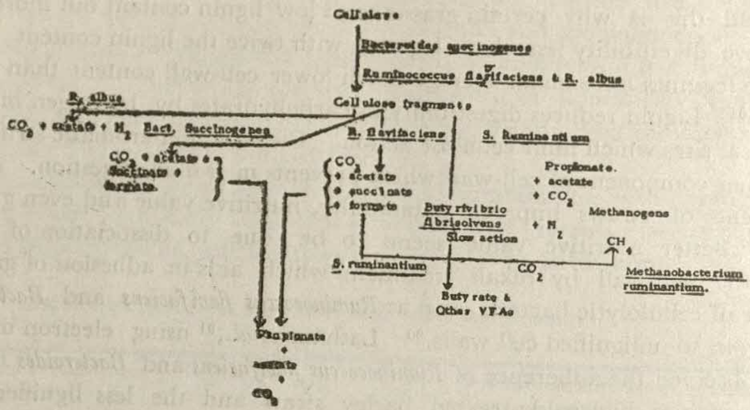

Cellulose
Bacteroides succinogenes
Ruminococcus flavifaciens & R. albus
Cellulose fragments
R. albus
CO$_2$ + acetate + H$_2$ Bact. Succinogenes
R. flavifaciens
S. Ruminantium
CO$_2$ + acetate + Succinate + formate.
CO$_2$ + acetate + succinate + formate
Propionate. + acetate + CO$_2$
Methanogens
+ H$_2$
Butyrivibrio fibrisolvens
Slow action
CH$_4$
S. ruminantium
CO$_2$
Methanobacterium ruminantium.
Butyrate & Other VFAs
Propionate + acetate + CO$_2$

Fig. 1. a.

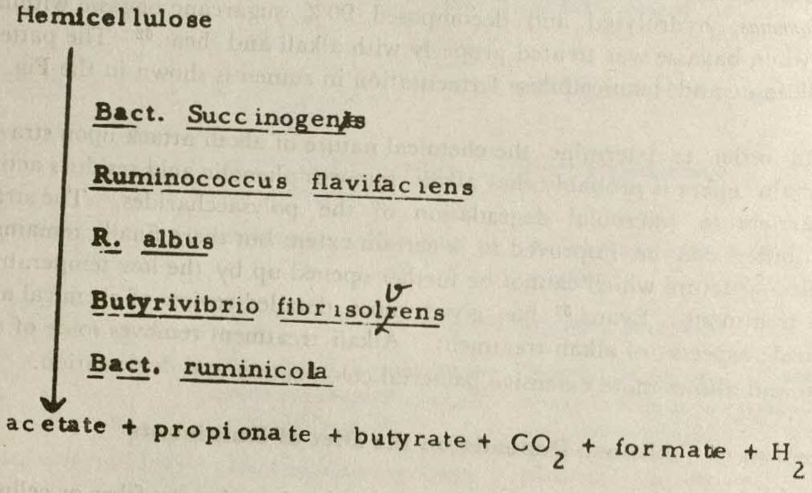

Hemicellulose
Bact. Succinogenes
Ruminococcus flavifaciens
R. albus
Butyrivibrio fibrisolvens
Bact. ruminicola
acetate + propionate + butyrate + CO$_2$ + formate + H$_2$

Fig. 1. b.

in vivo . el-Shazly *et al.*, [48] were able to show that the depression of purified-cellulose digestion caused by the addition of starch was the result of nitrogen limitation to the cellulolytic bacteria. Stewart [151] investigated the effect of pH on digestion of cotton yarn by rumen contents *in vitro*. When barley was added as a substrate to the fermentation, cellulose digestion was depressed, however when the pH of medium was maintained at 6.6, no depression occurred. Cellulolytic activity of rumen contents was found to be maximum at pH 7.0, decreasing to hear zero at pH 6.0. These studies suggest that depletion of noncarbohydrate nutrients, excess production of acid, or both could be responsible for the depression of cellulose digestion observed when feeding grain or starch to ruminants.

With roughage-type diets, the fermentation pattern is mostly through the multiplication of fibre-digesting organisms which digest cellulose and hemicellulose. The order of fermentation in rumen is glucose > xylose > starch > cellulose. Amorphous type crude fibre (Whatman filter paper) is attacked more readily than crystalline ones (cotton fibre). Bacterial concentration may be as high as 21 x 10^9 per ml of rumen liquor in Zebuand, approx. 25 x 10^9 per ml of rumen liquor in buffaloes.[128] There does not seem to be any difference in cellulose digestion rate.[81,82] The predominant bacterial species and their energy sources and fermentation products are given in Table 9.

Digestibility of Carbohydrates in Termites

The alimentary tract of termites consists of three main divisions: the foregut (Stomodeum), the midgut (Mesenteron) and the hindgut (Proctodeum). Its histology cytology and phylogenetic variations have been described in detail.[116,117] However, pertinent to this discussion is the location of the intestinal microbiota and sites of nutrient absorption. The clear bulk of the microbiota is housed in the hindgut (usually in dilated portion of the proctodeum known as paunch) which is also a major site of nutrient absorptions.[71]

Cellulose, hemicelluloses (xylans & mannans) undergo appreciable degradation on passage through the termite gut (65-99%)[49,173] and the assimilation efficiency of wood feeders is quite good (54-93%). Value for lignin digestion vary widely. The bulk of polysaccharide dissimilation occurs in the hindgut, and all available evidence indicates that the hindgut microbiota is the driving force of such activity.[24] In lower termite (families Nasotermitidae, Kalotermitidae, Hodotermitidae, Rhinotermitidae and Serritermitidae) which constitute about 25% of all species, the hindgut microbiota includes bacteria as well as unique genera and species of oxymonad, trichomonad and hypermastigote protozoa found almost howere else innature. Most of the protozoa present are cellulolytic anaerobes and appear to be the primary agents of cellulose decomposition. Wood particles passed to the hind gut are endocytosed by protozoa to CO_2, H_2 and acetate.

Table 9 Predominent rumen bacteria, their energy sources and their major fermentation products

Species	Energy sources	Major fermentation produce
Bacterioides Succinogens	glucose, cellulcose, cellobiase, strach	Acetate, succinate formate
Ruminococus albus	glucose, cellulose xylan	Acetate, lactate, formate, ethanol CO_2, H_2
Ruminococus flavefaciens	glucose, cellulose xylan	Acetate, succinate formate H_2
Ruminococcus fibriosolvens	glucose, cellulose xylan starch	Acetate, butyrate, CO_2 H_2 lactate, formate,
Bacteroides ruminicola	glucose, xylan, starch	Acetate, propoinate, succinate, formate
Bacteroides amylophilus	starch, maltose	Acetate, succinate, formate
Selenemonas ruminantium	glucose. starch, lactate glycerol, succinate	Acetate, propoinate, lactate, formate, CO_2
Stroptococcus bovis	glucose, starch	Lactate
Lachnospira	glucose, starch, pectin	Acetate lactate formate, ethanol, CO_2, H_2
Succinovibrio	glucose, dextrin	Acetate succinate, formate
Peptostrepto cocus elsdenii	glucose, glycerol lactate	acetate, propionate, butyrate, CO_2, H_2 caproic acid, valerate.
Vibrio Species	glycerol	Propionate
Methanobacterium ruminatium	formate, H_2	Methane

c. f. 104

The latter compound, after secretion from the protozoa, is absorbed from the hindgut and oxidized aerobically by termites for energy. Consistent with this scheme is the dominance of acetate in hindgut fluid of lower termites. Odelson & Breznak,[123] found that 94% of all volatile fatty acids in hindgut fluid of *Reticulotcrmes flavipes* was acetate, which was present in concentration of 74.7 mM. By using 14C labelled substrates, they were able to show that about 80% of acetate derives from cellulose carbon, whereas about 20% derives from hemicelluloses. Interestingly, acetate is also a dominant VFA in hindgut fluid of the higher termites *Nasutitermes nigriceps* and *Microtermes edentatus*.[87] Acetate is not only an oxidizable energy source for termites, it is an important precursor for synthesis of amino acids, cuticular hydrocarbons and terpens.

The abundance of streptococci in *Reticulitermes flavipes* hindguts, along with other bacteria that formed lactic acid in pure culture, suggests that lactate could be a significant fermentation product *in situ* and constitute an oxidizable energy source for the insect. Substrates for lactate formation might be xylose/or cellobiose which served as fermentable substrates for 145 streptococcal strains tested, and which could arise during hemicellulose or cellulose hydrolysis by other members of the biota (*e.g.* Protozoa).[148]

Mutual Relationship Between Gut Microbiota and Protozoa Helping Cellulose Digestion

A significant breakthrough has been made by Yamin[176,177,178] who achieved axenic cultivation of major cellulolytic protozoa species *in vitro*. The requirement for cellulose by *Trichomitopsis termopsidis* and *Trichomitopsis sphaerica* implied that cells possess cellulases and dissimilate the polysaccharide for energy. Both species fermented cellulose to CO_2, H_2 and acetate ; the same major products identified by Hungate, who used suspensions of protozoa harvested from termite hindguts. However, considering the extensive dissimilation of wood polysaccharides on passage through the gut, and the ability of protozoa to endocytose wood particles into food vacuoles, the protozoa probably hydrolyse hemicelluloses as well. Presumably, hydrolytic enzymes of these cells are quite effective at stripping wood polysaccharides from their complex with lignin. Yamin[177] and Hungate[75] found that 10-30% of the cellulose carbon fermented by protoza *in vitro* could not be recovered in acetate and CO_2. Yamin suggested that some of the missing carbon was present as soluble intermediates (oligomers) of cellulose hydrolysis released from the protozoa. It may be advantageous to protozoa to release hydrolysis intermediates *in situ*, since, if usable by bacteria, the protozoa could effectively culture a population of bacteria on which they might subsequently feed.

The requirement for killed rumen bacteria by axenic cultures, the mixed culture studies by Trager,[156] and the common occurrence of bacteria attached to (or with in) hindgut protozoa all lend credence to such speculation. O'Brien *et al.*[122] concluded that in the termites *Coptotermes lacteus* and *Nasutitermes exitiosus* synthesise their own cellulase, which in the case of lower termite *C. lacteus* is not sufficient to keep the insect alive when its protozoa may play a more important role in termite nutrition than simply digesting cellulose. Wood particles ingested by the symbiotic flagellates from *Reticulitermes lucifugus* are present in the cytoplasm and are not found in vacuoles.[92] This raises the possibility that the bacteria found in all xylophagous flagellates and not the flagellates themselves could be responsible for producing cellulolytic enzymes.

Role of Fungi in Cellulose Digestion

Fungi are found in the gut contents of termites but apparently do not contribute significantly to the digestion of cellulose.[73] Eutick *et al.*[50] found that feeding *Nasutitermes exitiousus* with the fungicide pimaricin had no effect on the life span of the termite. However. Rajgopal and Varwa[140,141] observed several gut fungi producing cellulose activiely solubilising cellulose and also enzyme responsible digestive cellobiose in the cell free medium.

Cellulose Digestion in Millepede and Cockroaches

Studies of some millepede species have indicated that cellulose is digested during passage through the intestinal tract[19,153]. However, the origin of cellulolytic enzymes is unknown. Taylor,[154] studied high numbers of bacteria able to grow on media containing cellulose, CMC, or cellobiose as the substrate were found in the alimentary tracts of the millepedes. Enzyme assay indicated that most cellulose and hemicellulose degradation occured in the midgut, whereas the hindgut was the site of pectin degradation. It appears that millepede-bacterium association is mutualistic and makes available to millepedes an otherwise mostly unutilizable substrate.

Studies by Wharton's group,[164] have demonstrated cellulases of both insect origin and bacterial origin in the alimentary tracts of several genera of cockroaches. Cruden and Markovetz,[43] isolated both obligately and facultatively anaerobic carboxymethyl cellulase (CMCase)-producing bacteria from the intestinal tracts of *Eublaberus posticus* and *Periplaneta americana*. The fermentation products of all the CMCase producing bacteria *i.e.* predominantly acetate and propionate, can either serve as substrates for other bacterial reactions or be transported across the wall of the hindgut of *P. americana*.

Fermentation of Cellulose in Human

Studies by Wolin,[169] showed that in the human large intestine the cellulose and hemicellulose of vegetables are fermented to acetic, propionic and butyric acids, just as they are in rumen. Wolin and Miller,[170] studied the VFA in faeces of healthy adults eating typical U.S. diets. It has been estimated that some human populations on very high fiber diets might obtain approximately 10% of their energy requirement from the VFA formed in the large intestine. Methane is also produced, but only in about one third of the adult population. Some individuals produce about 20% of the CH_4 per day than that a sheep produces. Individuals, who do not form CH_4, form H_2 gas. It was found that in faeces of methane forming adults in concentrations ranging from 1×10^7 to 3.2×10^{10}/g dry wt.[107]. There are also differences in fermentation reactions between rumen and human. For example, although species of Bacteroides are significant in the large intestine, they produce propionate from carbohydrate. In contrast, the rumen species produce succinate, and other genera decarboxylate the succinate to propionate. Interactions that depend on the transfer of H_2 to methanogens would not occur in humans who do not harbour methanogens.[170]

Digestion of Protein and Other Nitrogenous Compounds in Nature

Ruminant diets include 'protein' and 'protein supplement' which affect the degradation of protein to amino acid and further hydrolyzed to ammonia. This metabolic process is caused by a number of bacteria, protozoa present in rumen fluid and in rumen epithelial cells. Protein includes both essential and non-essential amino acids which undergo degradation by oxidative decarboxylation, mediated by ammonifiers.

Rate of protein digestion depends on solubility of protein[66] except bovine albumin which is water soluble protein but not easily digested.[6,98,120] About 38% of viable bacteria are proteolytic in nature. Blackburn *et al.*,[16] studied the degradation of protein nitrogen in sheep and redistribution of nitrogen after feeding and that the rate of digestion increased with the level of nitrogen nutrition increased. Thus diet positively effects the rate of proteolytic activity. Similarly protease activity in rumen fluid increased proportionately with the nutritional nitrogen levels.

Bacteroid amylophilus possess active proteinase activity. Strain *B. amylophilus* H 18 produce both extracellular (20% total activity) as well as intracellular protease activities.[18] *B. amylophilus* (R8/4) is known to be very active against leaf protein and nitrogen source.[63] *B. ruminicola, Butyrivibrio sp., Selenomonas ruminantum, Lachnospira multiperus* and *Peptostreptococcus elsdinii*[58] have the exopeptidase activity. Deamination and oxidative decarboxylation of peptides lead to liberation of ammonia. Another source of NH_3 is hydrolysis of urea which may of salivary or dietary origin. Protein content of milk increases with increasing concentration of urea in feed. Thus, the amino acid biosynthesis is, of great importance to the rumen microbial population and so to the ruminant itself.

Protein Biosynthesis

The rumen microbial population usually forms much of its amino acid from NH_3. Amino acid synthesis depends upon the rate of breakdown of ary nitrogen and the rate of absorption of NH_3. Quality of food protein is also important for microbial protein synthesis. Even with the normal diets, 30-80% of bacterial nitrogen and 25-64% of protozoal nitrogen may be derived from NH_3.[118] First NH_3 is taken up by the microbes from extracellular fluid, then the NH_3 is assimilated into amino acids, perhaps mediated by glutamine synthetase and the product of nitrogenase action is ammonia. Several microorganisms assimilate ammonia *via* the enzyme glutamic dehydrogenase (GDH), building it into glutamic acid from 2-oxoglutarate and thence into protein and other nitrogenous components of biomass :

$$\text{NAD(P) H} + NH_3 + \text{2 oxo glutarate} \xrightarrow{\text{GDH}} \text{NAD(P)} + \text{glutamate} + H_2O$$

Diazotrophic bacteria are very efficient in that the ammonia is assimilated into the cells as fast as it is formed and none escapes into the external environment. First, ammonia reacts with pre-existing glutamate to form glutamine, a reaction requiring ATP and mediated by glutamine synthetase (GS)

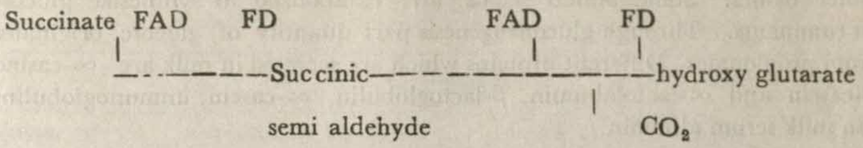
$$\text{glutamate} + NH_3 + \text{ATP} \xrightarrow{\text{GS}} \text{glutamine} + \text{ADP}$$

Secondly, the glutamine reacts with 2-oxoglutarate to form two molecules of glutamate, a reaction mediated by the enzyme glutamate synthase (GOGAT) :

$$\text{glutamine} + \text{2-oxoglutarate} + \text{NADPH} \xrightarrow{\text{GOGAT}} \text{2 glutamate} + \text{NADP}$$

Relation of Protein Metabolism with Carbohydrate Metabolism

Several microbes need volatile fatty acid plus ammonia or amino acid plus ammonia, for their growth. In a co-culture, *Bact. amylophilus* produce amino acids from ammonia and that amino acids were used by *Methansarcina elsdenii* and produce volatile fatty acid essential for *Ruminococcus albus*. The carbon skeletons could be obtained from excretion or break down of other organism and that total amino acid synthesis *de novo* did not present the growth of individual bacteria with specific nutritional requirements. Some volatile fatty acid derived from cellulose degradation produce amino acids.[3] Two enzymes isocitrate dehydrogenase and citrate synthase are essential for the synthesis of ∞ -ketoglutaric acid in Kreb's cycle by carboxylation of succinate with the help of *Bac. ruminicola*, *S. ruminatum* and *V. alcalescens*. These two enzymes are immediate precursor of glutamate dehydrogenase which makes glutamic acid. Some bacteria may form amino acids from volatile fatty acid *e.g.* valine from isobutyrate and isoleucine and leucine from ∝-methyl butyrate and iso valarate.[4]



$$\text{Succinate} \xrightarrow{\text{FAD} \quad \text{FD}} \text{Succinic semi aldehyde} \xrightarrow[\text{CO}_2]{\text{FAD} \quad \text{FD}} \text{hydroxy glutarate}$$

Degradation of Urea

Urecolytic *Staphylococcus*, *Microspora sp.*, *Propionibacterium cornybacterium* and strains of *Streptococcus* produce usease enzyme.[86] Another rumen fluid bacterium which is not referred a 'true rumen' microbe is *Streptofaecium* possesing high urease activity. It is estimated that about 5.8% of all the bacteria posses urecolytic property.

Digestion of Other Nitrogenous Compounds

Nitrate can be present in fairly large amounts in feeds stuffs. It is reduced rapidly to nitrite which quickly reduced to NH_3[163] failing which nitrite cause nitrite poisoning. *S. ruminantium* can use nitrate as a source of nitrogen for growth. Formate is good electron donor to prevent the formation of nitrite. Jones[83] reported microbial denitrification. ^{15}N incorporation into rumen microbes proved dinitrogen fixation, although acetylene reduction test showed low rates.[110] It was postulated that rumen nitrogenase derived from free-living nitrogen fixing microorganisms *via* feed. Nitrogen fixing *Bacillus macerans* was isolated from sheep rumen. However, by feeding molasses the N_2-fixation increased.[84]

Through the food, nucleic acids (RNA and DNA) enter the rumen (5.2-9.5% of total N). DNA and RFA are degraded by microbial enzymes to nucleotides, nucleosides, puriens and pyrimidines, uracial, hypoxanthine, xanthine and thymines. N-methylated amines, methylamine and tirmethylamines, derived from choline in remun, are hydrolyzed by *Metanosarcina barkerii*[129]. Purines and pyrimidines microbial degradation products are uric acids, allantoin and urea.

Adenine is converted to xanthine and hypoxanthine, xanthine further degraded along a pathway in which uric acid allantoin and allahtoate participates (Table 10).

Fate of Nitrogenous Compounds

The end products of digestion of protein and nucleic acid are NH^3. purines, pyrimidines and amino acids. Most of the ammonia is converted to urea in liver which is partly secreted. Urine is partly utilized to form glutamic acid, glutamine, carbonyl phosphate and the subsequet formation of pyrimidines and non-essential amino acids. Further, amino acids are absorbed and utilized for protein synthesis in the liver, kidney, mammary glands and other tissues. Some amino acids are catabolized to synthesize glucose in ruminants. Through gluconeogenesis part quantity of glucose originates from propionates. Different proteins which are secreted in milk are ∝-casino β-casein and ∝-lactolabumin, β-lactoglobulin, ∝-casein, immunoglobulins asn milk serum albumin.

Table. 10. Digestion of acids in small intestines

Enzymes	pH	Cleavage points	Type	Products
Pancratic ribonulease	7.7	Hodrolysis of phosphodiester	Endonuclease	Mono/polyribonucleotides
Pancreatic deoxyribo nuclease	6.8–8.2	Hydrolysis of phosphodiester bonds of DNA	endonuclease	mono-oligo-deoxyrichonucleotides
Intestinal phosphodiesterases I	9.3	End chain with 3' OH group	Exonuclease	nucleotides 5' -phosphates
Intestinal phosphodiesterases II	7.0	end chain with 5' OH group	exonuclease	nucleotides-3' -phosphates
Intestinal-5' nucleotidase	6.5	Hydrolysis of 5' nucleotides	–	nucteotides and phosphates.

Protein Digestion in Abomesum

Cow abomesum secretes 30-35 litres and sheep 5-6 litres of gastric juice in 24 hours. Gastric glands secrete HCl to keep digesta in acidic condition till pH fall to 2.00. Acidic conditions are needed for proteolysis of microbial protein and residual dietary protein by pepsin from peptic glands.

Protein Digestion in Small Intestines

Extra volatile fatty acids, minerals and vitamins which are left in rumen are absorbed in blood. Different proteolytic enzymes and their products are indicated in Table 11.

Protein Digestion in Large Intestines

The undigested food particles coming from small intestine come into large intestine which harbour *Bacteroides*, *Butyrivibrio*, *Fusobacterium*, *Streptococcus*, *Peptostreptococcus*, *Selenomonas* and *Micrococcus*. An over all schematic view of nitrogen cycle in ruminants is presented in Fig. 2.

Table. 11. Principal proteolytic enzymes and their amino acids specifications

Enzyme	Activator	Active enzyme	Optimum pH	Cleavage points
		Endopeptidase		
Tripsinogen	Enterokinase	trypsin	7.6 – 8.0	L arginine- L Lysine
Chymotripsinogen A&B	Trypsin	chymotrypsin A&B	7.8 – 8.0	L tyrosine- L tryptophan
Chymotryosinogen c	Trypsin	Chymotrypsine C	,,	" & L-methionine L-glutamine L-aspargine, L-valine, L
		Pancreatopeptidase E (Elastase)	8.8	leucine, L serine L-alanine
		Exopeptidase		
Carboxypeptidase A			7.5	All C-terminal amino acids except L-Lysine, L-arginine L-proline
Carboxypeptidase B			8.0	C-terminal, L-lysine, L-arginine

Physiology and Biochemistry of Digestion

Termites

Biochemical studies of termites have been carried out on the worker caste because they easily constitute the most numerous individuals in the nest and because of their importance in feeding the other species. In order to study the variaus physiological and biochemical reactions occurring in the termite gut, it is important to study the morphological and physiological state of the gut.

Condition in the gut

The digestive system of termites has been described in detail by Noirot and Noirot-Timothee[116] and extensively reviewed by O'Brien & Slaytor.[121] In summary, it consists of the foregut (consisting of the crop and gizzard), the midgut and the hindgut. The hindgut may be divided into five successive segments, the first proctodeal segment, the second segment called the eneteric valve, which controls entrance into the third segment known as the

The level of oxygenation of the gut was studied in a different manner by Bignell and Anderson.[42] They showed that when the guts of four species of termite (*Nasutitermes costalis*, *Cubitermes severus*, *Microcerotermes arboreus* and *Zootermopsis nevadensis*) were homogenized with air-saturated Ringer's solution was reduced, which they attributed to an oxygen deficit *in situ* in the gut and the consumption of oxygen in a chemical reaction. Table 12 shows the pH of the different sections of the gut of 16 termite species. The hindgut (including the paunch and colon) of most species has a pH in the range of 6-7.5 except for the soil feeding species (*C. severus* and *Procubitermes aburiensis* where conditions are more alkaline. Authors have also observed in *Odontotermes obesus*, the pH to be alkaline in the hindgut including the paunch.[127]

Thus the conditions in the hindgut are favourable for the growth of many microorganisms which are usually tolerant of pH values ranging from 6 to 9. The midgut, in which enzyme secretion occurs, also has a pH around neutrality.

Metabolism of Cellulose Biodegradation

The enzymatic hydrolysis of cellulose involves the action of two types of cellulase [1,4-(1,3:1,4) β-D-glucan-4-glucanhydrolase, EC 3.2 1.4], namely C_1 and Cx. The C_1 enzyme is active against crystalline or native cellulose and appears to be an exoglucanase whereas the Cx cellulose hydrolyses non-crystalline cellulose or soluble cellulose derivatives (for example, carboxymethyl cellulose) and appears to be an endoglucanase. A third enzyme, cellobiase or β-glucosidase (β-D-glucoside glucohydrolase, Ec 3.2. 1.21), which hydrolyses cellobiose to glucose.[174] The increased effectiveness of mixtures of endo and exogluconases and β-glucosidase on native cellulose hydrolysis,[147] may be due to the exoglucanase preventing the reformation of glycosidic bonds following endogluconase cleavage. To facilitate efficient depolymerization, exo-and-endogluconases may even combine in an enzyme complex.

Role of Enzymes in Cellulose Digestion

Higher Termites

In a study of the Cx-cellulase activity of *Trinervitermes trinervoides* Potts and Hewits[136] found that about 70% of the total activity was in the midgut and that about 40% of this activity was associated in the midgut wall. In one experiment, the hindgut was shown to possess about 15% of the cellulase activity, whereas in another experiment a value of about 50% was obtained. Potts and Hewitt[136] were unable to remove the gut flora of *T. trinervoides* with antibiotics, but still concluded that the cellulase was produced by the termite itself since no bacteria was found in the foregut and midgut. The partially purified cellulase showed both C and Cx characteristics since it was capable of hydrolysing crystalline cellulose and carboxymethylcellulose.

Table. 12. pH of the guts of termites

Species	Crop	Foregut	Midgut	Mixed segment	Hindgut	Paunch	Colon	Rectum
Kalotermes flavicollis		5.2-5.4	6.8-7.5		5.0-7.5			
Anacanthotermes ahngeriamus		7.7	6.9		7.9			
Anacantnotermes turkestanicus					7.7			
Zootermopsis angusticollis		5.2-6.8	5.2		3.0-6.8			
Zootermopsis nevadensis	6.5-7.0		7.0-7.5			7.0-7.5		7.0-7.5
Reticulitermes lucifugus	5.5-6.0		6.5-7.0			6.0-6.5		6.5-7.0
Reticulitermes lucifugus		5.6	6.8-7.2		7.8	7.4[1]		
Reticulitermes sp.		5.5			5.0			
Coptotermes lacteus		3.8-4.4	6.8-7.4			6.8-7.4	6.8-7.4	2.8-3.2
Microcerotermes edentatas		8.8-9.6	8.8-9.6	9.6	6.0-9.6	7.2-7.6	6.8-7.2	6.0-6.8
Microcerotermes arboreus	6.5-7.5		7.0-8.0	7.5-8.0		6.4-7.0	6.5-7.0	6.5-7.0
Microcerotermes sp.					8.3			
Cubitermes severus	6.0- 6.8		6.5- 7.5	8.0 9.5		9.0- 10.0	7.5- 9.0	7.0- 8.0
Procubitermes aburiensis	6.0- 6.5		6.5- 7.5	8.0- 9.0		9.0- 9.5	7.5- 8.5	7.0- 8.0
Nasutitermes costails	5.5- 6.0		5.5- 6.0	8.0- 8.5		5.5- 6.5	6.5- 7.0	6.5- 7.0
Nasutitermes exitiosus		2.0- 2.8	6.8- 7.5	7.0- 7.5		about 6.8	about 6.8	about 2.8- 3.8
Trinerviterms trinervoides			5.6					

c. f. R. W. O'Brien and M. Slaytor [121]

The cellulase activity of *Termes obesus (Cyclotermes)* resided wholly in the hindgut.[109] These authors isolated cellulase degrading bacteria (Coryneforms) which, however, did not increase the rate of cellulose digestion when mixed with gut extracts.

O'Brien *et al.* [122] found that the Cx-cellulose of *Nasutitermes exitiosus* was distributed in the foregut (19%), midgut (59%) and mixed segment (14%) and the hindgut (8%). Removal of the gut flora by feeding the termite with tetracycline or by starving the termite did not affect the activity of the total cellulose, indicating that the termite secreted its own enzymes.

A major product of the hydrolysis of cellulase by cellulase is the disaccharide cellobiose, which in turn can be hydrolysed to glucose by cellobiase or β-glucosidase. Cellobiase was evenly distributed between the midgut and hindgut paunch of *Trinervitermes trinervioids* the cellobiase activity in the hindgut was attributed to the hstndgut bacteria.[136] Kovoor[88] found considerable variation cellobiase activity in *Microtermes edentatus* with workers from one nest showing activity in the midgut, whereas those from another nest showed actitity in both the hindgut and midgut. In *Macrotermes natalensis*, β-glucosidase activity was mainly in the midgut,[99,100] and in *M. subhyalinus* the activity was distributed equally between the midgut and the paunch.[2] In *Termes obesus (cyclotermes)*, cellobiase was mainly in the hindgut.[109] Over 90% of the cellobiase activity of *Nasutitermes exitosus* and *N. walkeri* was located in the midgut.[106]

Lower Termites

Yokoe[170], using *Reticuliermes speratus*, a lower termite, concluded that at least a part of the cellulase (C_x) was secreted by the termite. It was based on a finding that defaunated termites and nymphs (devoid of Protozoa) showed cellulase activity. In a later study with the same termite species, Yamaoka and Nagatani[175] found that extracts of salivary glands contained C_1 and C_x-cellulase activities, whereas only C_x-cellulase was detected in extracts of the foregut and midgut. Extracts of the hindgut protozoa contained C_1 and C_x cellulases.

C_x-cellulase actsvity in *Hodotermes mossambicus* was distributed through all sections of the gut of the workers, but was mainly in the first paunch of the larvae and the second paunch of the soldiers.[20,143] On feeding the termite with green leaves, a decline in the protozoal population was observed but an increase in C_x-cellulase activity upto six fold was observed in the combined extracts of the foregut and midgut which are essentially free of protozoa. These results indicate that the termites can secrete C_x-cellulase and the amount of enzyme secreted is atleast half of the total cellulase activity of whole termites. The C_x-cellulase activity in the foregut and midgut of *Coptotermes lacteus* was unaffected by treatments which killed the hindgut protozoa.[122] McEwan *et al*[106], found 78% of the cellobiase in the hindgut and 21% in the midgut. Removal of gut protozoa and spirochaetes by oxygen treatment did not affect the midgut activity, but resulted in almost the complete loss of the hindgut activity. The protozoa in the hindgut had a high cellobiase activity which accounted for most of the activity of this enzymes found in the hindgut. The cellobiase of *Mastotermes darwiniensis* was present in the salivary glands (37%), midgut (37%) and hindgut (20%).[159]

In Millipede

In the study of the cellulose digestion by Taylor (1982) in desert millipedes *Orthoporus ornatus* and *Comanchelus sp.* enzyme assays indicated that most cellulose and hemicellulose degradation occurred in the midgut, whereas the hindgut was an important site for pectin degradation. Hemicellulase and β-glucosidase in both species and possibly Cx-cellulase and pectinase in *O. ornatus* were of possible microbial origin. Degradation of (14c)cellulose by millipedes whose gut floras were reduced by antibiotic treatment and starvation demonstrated a reduction in 14 CO_2 release and 14_c assimilation and an increase in 4c excretion over values for controls.

Role of Fungus in Enzyme Secretion

The macrotermitinae have a symbiotic relationship with fungi of the genus *Termitomyces*. These termites cultivate fungus gardens by growing fungi on structures known as 'fungus combs' which are derived from chewed, but undigested plant fragments or are constructed from faecal pellets.[94] The conidiophores appear as white nodules on the surface of the comb and are eaten along with comb material, by the termites. The termites starve if deprived of the fungus comb and the conidiophores.[145]

The fungus nodules possess both C_1 and Cx-cellulases and studies with *Macrotermes subhyalinus* and with *M. natalensis*[99,100] have led to the hypothesis that these termites acquire most of their C_1 cellulase by ingesting the conidiophores. C_1-cellulase activity in the salivary glands and the paunch of *M. natalensis* was very low (0.2–0.6/μ mol of reducing euqivalents measured as maltose per minute per termite) but was of reasonable activity in the midgut (7.6+_ 0.8 μ moles of reducing equivalents per minute per termite), whereas a high activity of Cx-cellulase (about 10-32 μ mol of reducing equivalents per minute per termite), was found in the salivary glands, the midgut and the paunch.[99,100] In termites which are either deprived of the nodules or were starved, the activity of the midgut C_1-cellulase declined, whereas the Cx-cellulase remained constant; C_1 and Cx cellulase activities remained high in those termites fed on the nodules.

Most of the C_1-cellulase in *M. subhyalinus* was found in the contents of the midgut and only about 3% was associated with the midgut wall, whereas some 33% of Cx-cellulase was present in the midgut wall, indicating that little C_1–cellulase is secreted by the termite.[8] The distribution of enzymes in the paunch was 19% for C_1-cellulase, and 43% for C_x-cellulase. Practically no or very little C_1-cellulase activity was observed in termites raised on a fungus-free diet, although traces of C_x-cellulase were found. Starvation of the termites caused a 50% decline in C_1-cellulase and a 20% decline in C_x-cellulase.[3] The results of Martin and and Martin[99] explain that the nutritional dependence upon the fungus nodules as a source of the critical G_1-enzymes, which must be acquired before they are able to effect the digestion of cellulosic materials.

In studying cellulase activity in termites most investigators have used some soluble form of cellulose usually carboxymethyl cellulose, as a substrate and thus have measured only C_x-cellulase activity. The use of crystalline cellulase as a substrate has been restricted to a few studies and has indicated low activities of C_1-cellulase in the salivary glands of *Reticulitermes speratus*,[175] *Macrotermes natalensis*[99],[100] and *Masotermes darwiniensis*.[159] The activity of C_1-cellulase is very low when compared to the Cx cellulase activity found in these termites, but may simply be a reflection of the insoluble nature of the substrate used to mearure C_1-cellulase activity. However, there is little evidence for the synergistic action of C_1 and Cx-cellulases in certain fungi and for Cx-cellulase and not C_1-cellulase, being responsibie for the initial attack on crystalline cellulose.[173]

Rumen Environment

Conditions suitable for microbial growth, particularly with respect of residence times of digests, need to be provided in the digestive tract. These are obtained in some cases by an enlargement of the large intestine, where microbial action takes place. In the ruminant herbivores and some related animals, a separate organ has evolved in which microbial action takes place before the gastric digestion (Fig.4).

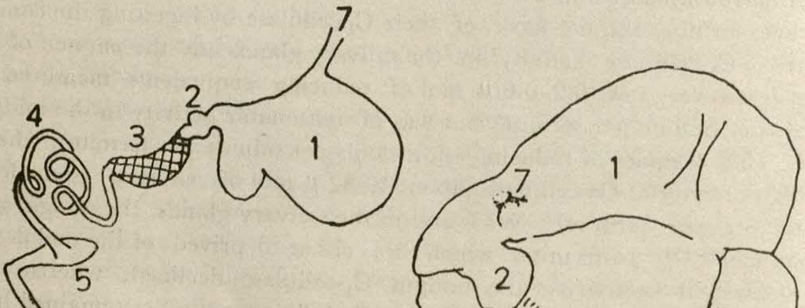


Fig. 4.

The environment in rumen is completely anaerobic and microorganisms best suited and adopted to it are found. The temperature in the rumen is 38°-42°C. The pH is more or less steady, and is maintained by the aborption of volatile fatty acids and ammonia. The saliva pouring into the rumen helps to buffer and maintain pH at 6.8 due to high HCO_3 and PO_4 ions. Such an environment in rumen leads to a very low oxidation and reduction potential (Eh = −300 to −400mV). Gas phase constitutes 50–70 per cent CO_2 and the rest is methane. During the act of eating, a litte oxygen gets into the rumen with feed which very rapidly metabolized.

Enzymes Responsible for Fiber Digestion in Rumen

Number of enzymes isolated from mixed and single rumen organism are able to hydrolyse preparations of the polysaccharides. The substrates used to study the digestion are prepared either commercially or from plant material and this involves physical or chemical (or both) treatments that will result in some change in the structure of the molecule from that of the intact plant. The ability of enzymes to attack these substances may not be entirely indicative of their attack on intact feed stuff substrates. However, it is difficult to overcome such problems.

Earlier work on enzymes have been reviewed by Prins[139]. The work discussed previously has shown that the cellulolytic bacteria, in general, attack both cellulose and hemicellulose in plant cell walls, Pettipher and Latham,[132] investigated in detail the fiber-digesting enzymes of a strain of *Ruminococcus flavefacien*. In culture supernatants of bacteria grown with cellobiose or filter paper as the energy source, enzymic activity against the following substrates was detected. The activities are expressed as total reducing sugar released from each substrate with ball-milled filter paper is given in Table 13.

Table 13. Cellulase activity using diffrent substrates

Substrates	Activity expressed as total reducing sugar*
1. Larch-wood xylan	9.9
2. Oak sapwood hemicellulose	5.8
3. Ryegrass mesophyll cell walls	3.4
4. Carboxymethyl cellulose	9.3
5. Filter paper	1.0
6. Solka-floc	0.5
7. Avicel	0.4
8. Scoured native cotton	0.03

*ball-milled filter paper, arbitrarily assigned the value 1, c. f. Hobson, P. N. and Wallace, R. J. 1982. Microbial ecology and activities in the rumen Part I CRC Critical Reviews in Microbiology pp. 188.

The experimental data indicate that enzymes, commensurate with the properties of the bacteria, are liberated into the culture medium ;and so could be liberated into plant cells (used as feed) close to the bacteria in the rumen. The authors also emphasize the fact that bacteria can utilize various natural and synthetic substrates. The cellulase, xylanse and aryl β-glucosidase all had pH optima at 6.4 about the normal rumen pH when forages are fed, and temperature optima of 39 to 45°C. A cellulolytic enzyme mixture from rumen cotents had pH optima of 6.9.

The pH optima of the enzymes is related to the pH at which the bacteria can grow. For instance, Stewart [151] had previously found that reducing the pH or rumen contents to 6.0 from 7.0, almost completely destroyed cellulolytic activity and reduced the numbers af culturable cellulolytic bacteria. The effect of addition to the feed of starch, such as barley, on lowering digestion of fibres seemed from this study to be mainly an effect of the lowering of rumen pH by rapid fermentation of starch. Gel filtration showed that although the enzymes were recovered from the culture supernatant, a large proportion of the polysaccharase activities was associated with a high molecular weight that could have originated in the bacterial cell wall.[132] Yu and Hungate[180] examined extracellular cellulolytic enzymes from culture fluids of a number of strains of *R. albus.* At least four cellulases, differing in molecular weight were found, but strains varied in cellulease activities. The soluble prodcts of hpdrolysis of cellulosic substances differed for the four enzymes.

In all three cellulolytic rumen bacterial species of *Ruminococcus albus*, *Ruminococcus flavifaciens* and *Bacteroides succinogens*, Hiltner Dehority [68] observed that the only effect of soluble carbohydrates on the rate and extent of cellulose digstion appeared to be an inhibition resulting from a low pH generated by the rapid fermentation of soluble carbohydrates. These results support the concept that cellobiose and glucose do not repress cellulase activity in intact bacteria.

Energy Value

Microorganisms ferment and convert large quantities of carbohydrate components into volatile fatty acids which yield considerable energy in the form of ATP. Cellulose catabolism into cellobiose by cellulase serected by mixed cultures of rumen microorgansim by random cleavage is well recognized[119]. Further hydrolytic split of cellobiose by *Ruminococcus flavifaciens* or *R. albus* is known as the process of phosphorylytic cleavage which leads to the liberation af high energy bonds[7,78].

$$\text{Cellobiosc}+\text{Pi}\text{—}\xrightarrow[\text{H}_2\text{O}]{\text{B glucosidase}}\text{Glucose}\cdot 1\text{-PO}_4+\text{Glucose}$$

Starch molecule is also randomly split the same way as cellulose by amylase into maltose liberated by rumen bacteria and protozoa.[69] Maltose is further broken down by an enzyme microbial maltose phosphorylase to liberate glucose–PO_4.

$$\text{Maltose}+\text{Pi} \xrightarrow[\text{Phosphorylase}]{\text{maltose}} \text{Glucose} - 1\text{–}PO_4+\text{Glucose}+H_2O$$

Xylan is depolymerized to xylo-oligosaccharides, xylobiose and xylose.[161] Pentoses are thereafter fermented to hexose monophosphates to the extent of 30% through the action of trandolase and transketolase enzymes.[72] Hexose monophosphates are later converted into pyruvic acid in the rumen through normal Embden-Myerhoff pathway (Fig. 5) giving a net gain of 3 ATP.[8] Microbes convert pyruvate to acetyl-co-A or acetyle phosphate. The ultimate endproducts are volatile fatty acids (Fig. 6).

Pyruvate Decarboxylation Mechanism

Clostrial phosphoroclastic type reaction (Thioclastic system) requires co-enzyme A, Fe as ferredoxin and diphosphothiamine (DPT) as co-factors to catalyse the oxidative decarboxylation of pyruvate to form acetyl phosphate, CO_2 and H_2 (Wolfe and O'Kave[168]) (Fig. 7).

The rumen organisms causing this type of reaction are *Clostridium butyricum* and *Peptostreptococcus elsdenii*. Coli-aerogens phosphoclastic-type reaction (formate phosphoroclastic system) requires CoA-5H, DPT, Fe^{++} and possibly Mn^{++} or Mg^{++}. The products are acetyl phosphate and formate.

$$CH_3COCOOH+PO_4 \xrightarrow[Mn^{++},Fe^{++}]{\text{CaA–SH.DPT}} HCOOH+\text{acetyl-}PO_4$$

This type of reaction has been characterised in *Veillonella alcalescens* under alkaline conditions and under many rumen cellulolytic organisms. They liberate formate and not hydrogen as one of the products (Pazur *et al.*[130]) which is rapidly converted into CO_2,H_2 and methane in the rumen by two methods (Brill *et al.*[26]) (Fig. 8).

Methanogenesis

Volatile fatty acids and methane are the major reduced products of anaerobic fermentation in rumen and caecum. The liberation of volatile fatty acids is an energy gain and that of methane an energy loss.[10] which amounts to about 8% of the total energy in feed consumed. Though energy released during methane formation is utilized by microbes, yet the host animal can use it more efficiently if the electrons utilized in methane formation can be diverted to other reduced products such as volatile fatty acids.[27]

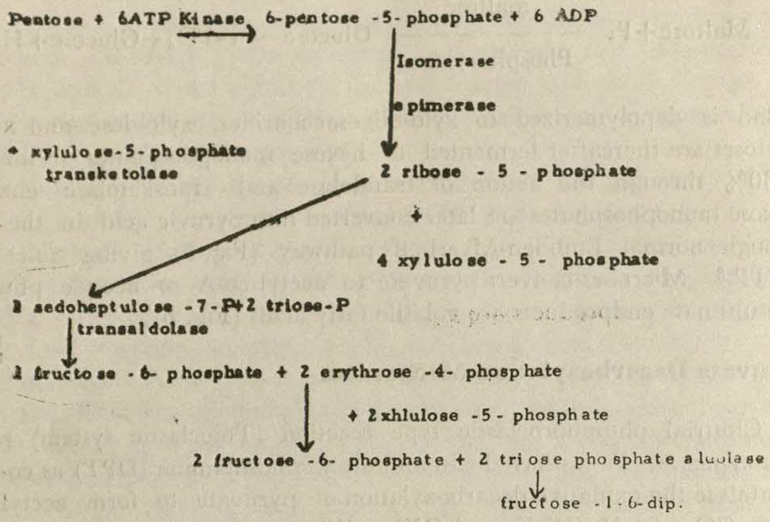

Pentose + 6ATP Kinase 6-pentose -5- phosphate + 6 ADP
Isomerase
epimerase
+ xylulose-5-phosphate
transketolase
2 ribose - 5 - phosphate
+
4 xylulose - 5 - phosphate
2 sedoheptulose -7-P+2 triose-P
transaldolase
2 fructose -6- phosphate + 2 erythrose -4- phosphate
+ 2 xhlulose -5 - phosphate
2 fructose -6- phosphate + 2 triose phosphate aluolase
fructose -1·6-dip.

Fig. 5

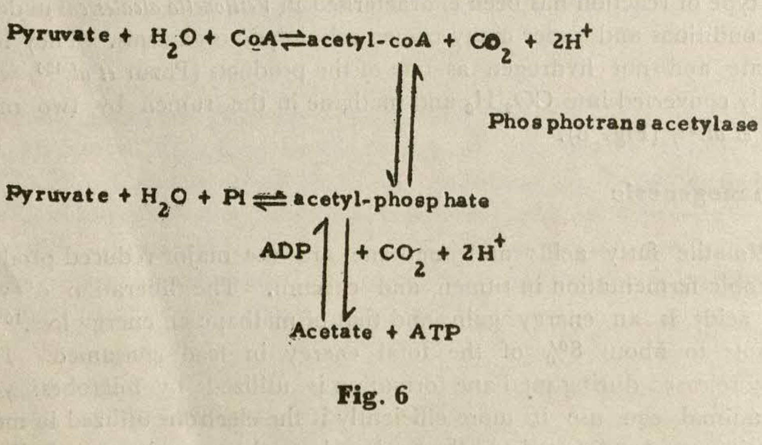

Pyruvate + H_2O + CoA ⇌ acetyl-coA + CO_2 + $2H^+$
Phosphotransacetylase
Pyruvate + H_2O + Pi ⇌ acetyl-phosphate
ADP
+ CO_2 + $2H^+$
Acetate + ATP

Fig. 6

$$CH_3COOH + CoA - SH^{+}$$

$$\xrightarrow[Fe^{++}]{FDPO_4 \quad DPT} CO_2 + \text{acetyl} - CoA + FDH_2$$

$$FDH_2 \xrightarrow{\text{hydrogenase}} H_2 + FD.$$

or

$$FDH_3 \xrightarrow[\text{DPN reductase}]{+DPN} DPNH + FD.$$

$$\text{acetyl-}CoA + ADP \xrightarrow[\text{Phosphotransacetylase}]{} \text{acetyl-}PO_4 + CoA - SH$$

$$\text{Acetyl} - PO_4 + ADP \longrightarrow ATP + CH_3COOH$$

Fig. 7

1. $HCOOH \xrightarrow[\text{dehydrogenase}]{\text{formic}} CO_2 + H_2$

2. $HCOOH + FD \xrightarrow[\text{dehydrogenase}]{\text{formic}} CO_2 + FDH_2$

$$FDH_2 \xrightarrow{DPN} FD + DPNH$$

Fig. 8

CO_2+4H_2————————————CH_4+2H_2O
$CH_3+H^++e^-$——————————CH_4
$HCOOH$————————————CO_2+H_2

Rumen methane bacteria requires 2-methyl butyrate, ammonia ions, acetate and other factors in rumen fluid to stimulate their growth.[67] Different individuals of the same species and even with similar type of diets produce methane at different rates.[45] Decrease in methane production is usually favourable to higher propionate production, a distinct advantage to the host animal.[77] Depression in methanogenesis can be brought about by decrease in dietary fibre content or reduction in particle size of the feed.

Methanogenic bacteria grown on hydrogen plus carbondioxide as sole energy source.[57] Some of them, including *Methanobacterium thermoautotrophicum* can use hydrogen plus carbondioxide as sole carbon source. Methanogenic bacteria take up hydrogen *via* an active hydrogenase which is specific for factor 420 as electron acceptor. Factor 420 functions as central electron donor for CO_2 reduction to CH_4 and for CO_2 assimilation into cell carbon. NADP reductase, pyruvate sunthetase and ∝-Keto glutarate synthase have been found to be specific for factor 420 (Fig. 9).

Minerals and Rumen Microbes

Several inorganic minerals like Sulfur, selenium, zinc, cobalt, sodium, copper and silica play an important role in accelerating the metabolic activity of rumen microbes.

The biological sulfur cycle is no exception to this generalization: Microbes play a major quantitative role in the cycling of sulfur in the rumen biosphere and it's fermentation products. The sulfur metabolism is classified into organic system in which it is utilized in the rumen to synthesize-sulfur amino acids and the inorganic system in which it is utilized in the form of active sulfate. Great number of soil, water, intestinal and sewage bacteria can decompose organic matter, the waste products of living things, and in many instances the main Material being decomposed is protein. Two of the main amino-acids constituting proteins contain the sulfur atom: methionine ($CH_3.S.CH_2.CH._2CHNH_2.CO_2H$) and crysteine ($HS.CH_2.CHNH_2.COOH$). Cystine, the disulfide dimer of cysteine, is considered as formally equivalent to cysteine for present purposes. Bacterial degradation of protein usually leads to release of degradation products which have a free thiol group (*e.g.* cysteine, homocysteine) and these are decomposed by enzyme called 'desulfhydrases'. A typical example is the enzyme cysteine desulfhydrase which removes the amino and sulfhydryl groups from cysteine, probably in that order:

$$\underset{\text{Cysteine}}{HS.CH_2.CH.NH._2CO_2H} \xrightarrow{-NH_3} \underset{\beta\text{-mercap to pyruvic acid}}{HS,CH_2.CO.CO_2H} \xrightarrow{-H_2S} \underset{\text{pyruvic acid}}{CH_3.CO.CO_2H}$$

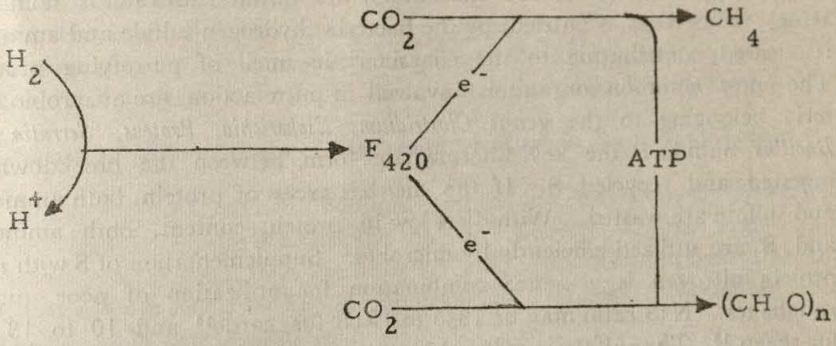

CO_2
CH_4
H_2
e^-
F_{420}
ATP
H^+
e^-
CO_2
$(CH_2O)_n$

Fig. 9

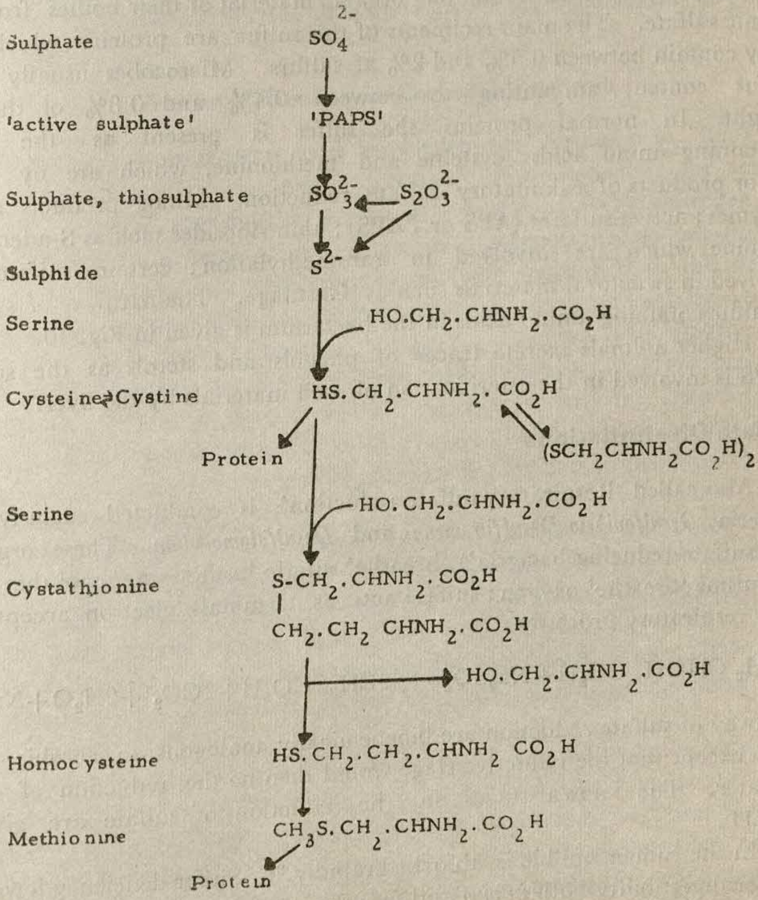

Sulphate
SO_4^{2-}
'active sulphate'
'PAPS'
Sulphate, thiosulphate
SO_3^{2-}
$S_2O_3^{2-}$
Sulphide
S^{2-}
Serine
HO.CH$_2$.CHNH$_2$.CO$_2$H
Cysteine⇌Cystine
HS.CH$_2$.CHNH$_2$.CO$_2$H
Protein
(SCH$_2$CHNH$_2$CO$_2$H)$_2$
Serine
HO.CH$_2$.CHNH$_2$.CO$_2$H
Cystathionine
S-CH$_2$.CHNH$_2$.CO$_2$H
CH$_2$.CH$_2$ CHNH$_2$.CO$_2$H
HO.CH$_2$.CHNH$_2$.CO$_2$H
Homocysteine
HS.CH$_2$.CH$_2$.CHNH$_2$ CO$_2$H
Methionine
CH$_3$S.CH$_2$.CHNH$_2$.CO$_2$H
Protein

Fig. 10

The pyruvate (or α-Ketoglutarate if the initial substrate is homocysteine) is used as a nutrient by the bacteria, hydrogen sulfide and ammonia is released, contributing to the characteristic smell of putrefying protein. The most common organisms involved in putrefaction are anaerobic bacteria belonging to the genus *Clostridium*, *Escherichia*, *Proteus*, *Serratia* and *Bacillus*. Sulfide is the key intermediate form between the breakdown of ingested and recycled S. If the diet has excess of protein, both ammonia and sulfide are wasted. With diets low in protein content, both ammonia and S are utilized efficiently by microbes. Supplementation of S with non-proiein nitrogen is a better combination for utilization of poor quality roughages. N :S ratio may be 13 :5 to 15 :1 for cattle[14] and 10 to 13.5 :1 for sheep.[13] The sulfate is reduced by microorganisms in two main ways :

Sulfate Assimilation

Assimilatory microbes, but not genrally speaking multicellular animals, can build up the sulfer-containing organic material of their bodies from inorganic sulfate. The main recipients of this sulfur are proteins which generally contain between 0.5% and 2% af sulfurs. Microcobes usually have a sulfur content amounting to between 0.4% and 0.8% of their dry weight. In normal proteins the sulfer is present as the sulfer-containing amino acids cysteine and methionine, which are by far the major products of assimilatory sulfate reduction. Minor products are co-enzymes ; active sulfates (APS or PAPS) ; thio-ribosides such as S-adenosymethionine which are involved in transmethylation ; certain sulfate esters involved in structural materials such as Cartilage. The pathway of synthesis of sulfur-cotaining amino acids in the bacterium is given in Fig. 10.

Higher animals excrete traces of phenols and sterols as the sulfates ; PAPS is involved in the esterification of such materials by the liver.

Sulfate Dissimilations

Also called 'Respiratory sulfate reduction', is conducted exclusively by bacteria, *Desulfovibrio Desulfuromenas* and *Desulfotomaculum*. These organisms (the sulfate-reducing bacteria') 'breathe' sulfate in the same sense that higher organisms 'breathe' oxygen : sulfate acts as terminals electron acceptor for their respiratory processes.

$$(2CH_3\ CHOH,\ CO_2H+Na_2SO_4——2CH_3.\ CO_2H+2CO_2+2H_2O+Na_2S)$$

Pathway of sulfate reduction are biochemically analogous to aerobic respiration except that the reductive stage would then be the reduction of oxyzen to water. The known stages in the reduction of sulfate are given in Fig. 11.

In the rumen, sulfide is absorbed rapidly.[21] Sulfer deficiency lowers dry matter digestibility both *in vivo* and *in vitro*. Sulfide formed in the rumen is either converted into microbial protein or is absorbed by the rumen wall.

Absorbed sulfide is oxidized to sulfate in blood and liver and thereafter disributed into the extracellular fluid. Sulfate is recycled directly to the rumen *via* the salivary secretion or recycled to large intestine providing a conservative mechanisms with feeds low in sulfer.[85] Sulfur cycle in rumen is given in fig. 12.

Effect of Other Mineral Elements on Rumen Bacterial Action

Selenium, zinc, cobalt, Sodium, copper are metabolized by rumen bacteria for synthesizing proteins and vit. B_{12}. Sodium, copper and cobalt improve the digestibility of crude fibre[146]. Nickel has been implicated as the metal component of Factor F. 420 responsible for methane production from *Methanosarcina thermoautotrophicum*.

Silica in the form of silicates occur in microbes and plant materials. The legumes contain lesser quantity of silicates as compared to those in non-legumes. It is a structural element of cell-wall as organic complex and functions just like lignin in fodders. Silica occurs in fodder in two forms; one is soluble and the other insolube saluble silica lowers the digestibilility of fodder organic matter.

Nitrozen Fixation in Gut and Rumens

The termite mounds were used by farmers as fertilizer in the initial stages of agricultural development. Kozlova[89] estimated that the termite mound soil (500/ha) contained 47 Kg/ha of readily available nitrogen and considered that the favourable conditions of temperature and moisture in mounds favoured rapid mineralization of nitrogen contained in the organic matter. The termite mounds and other structure contained relative to the surrounding soil, morc Carbon, Nitrogen, Calcium and slightly more Potassium.[94] Termites can live for considerably long time on low protein and nitrogen diet. They can fix atmospheric nitrogen,[22] with the help of associated gut symbiotic bacteria. A more positive biochemical proof for accurence of nitrogen fixing enzyme-nitrogenase was provided from *Rhynxchotermes* and *Nasutitermes* species.[137]

In Table 14, the inferred rates of nitrogen fixation have been correlated to TDN equivalent which is the time it would take for termite to double their nitrogen content if $N = N$ fixation rate is constant, and if atmospheric dinitrogen was the sole source of nitrogen. The dinitrogen fixation would seem very important to the *Nasutiterms* species and others whose TDN value ore relatively low. *Enterobacter agglomeras* is postulated to be presumptive nitrogen fixing microbe.

Diazotrophic bacteria have been reported from the excreta of humans and guineapig[11] and pigs.[133] They are present in the rumens of cattle, sheep and deer.[70] *Desulfotomaculum ruminis*, a diazotroph has been reported from a sheep rumen. Oomen[125] has observed a pronounced negative nitrogen balance among certain natives of New Guinea, whose diet is particularly rich in carbohydrate (as sweet potatoes) and low in nitrogen and has suggested

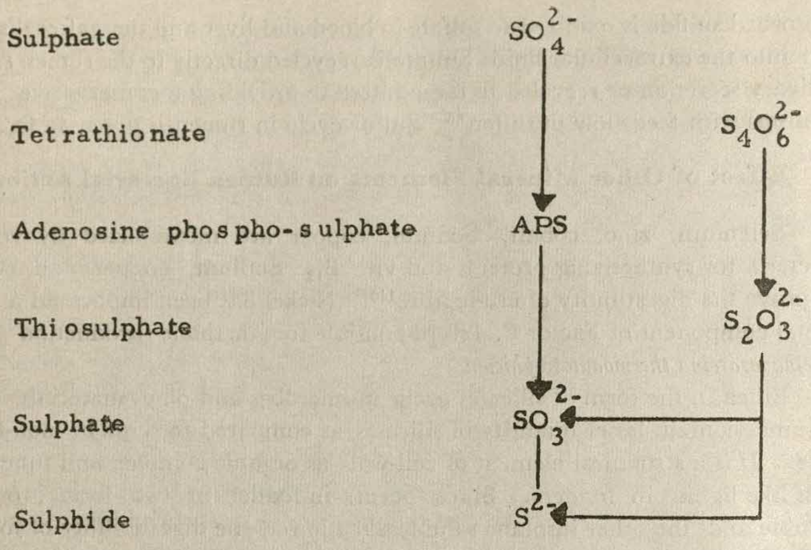

Sulphate
Tetrathionate
Adenosine phospho-sulphate
Thiosulphate
Sulphate
Sulphide
SO_4^{2-}
$S_4O_6^{2-}$
APS
$S_2O_3^{2-}$
SO_3^{2-}
S^{2-}

Fig 11

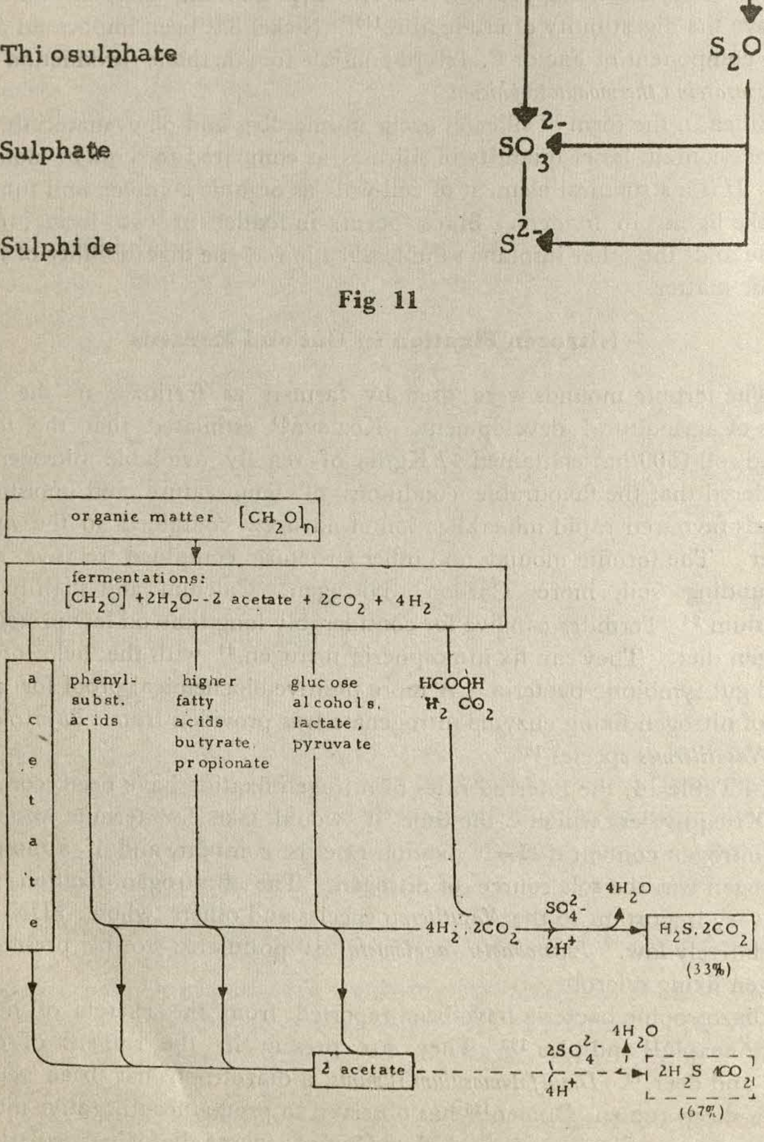

organic matter $[CH_2O]_n$
fermentations:
$[CH_2O] + 2H_2O \text{--} 2$ acetate $+ 2CO_2 + 4H_2$
acetate
phenyl-subst. acids
higher fatty acids butyrate propionate
glucose alcohols, lactate, pyruvate
HCOOH
H_2 CO_2
$4H_2$, $2CO_2$
SO_4^{2-}
$2H^+$
$4H_2O$
$H_2S, 2CO_2$
(33%)
2 acetate
$2SO_4^{2-}$
$4H^+$
$4H_2O$
$2H_2S$ $4CO_2$
(67%)

Fig. 12

Table 14. N fixation (Acetylene reduction) in live termites.

Termites 1	Caste 2	Bg N fixed/ b(fresh wt X day)T 3	IDN(cx)g 4
Amitermes sp.	W	0.36	126
	S	0.45	100
Armiterms sp.	W+S	1.44	31
Coptotermas formosanus	W	0.16 49.39	283-1
		0.03	1,507
C. lacteus	W	0.37-187	122.24
Cornitermes sp.	W+S	1.06	43
Cryptotermes brevis	BL	0.38	119
Cubitermes sp.	W	0.17	265
	R	0.00	
Heterotermes sp.	W	0.94	48
	S	0.00	
Incitsitermes minor	W	1.00 18.87	45-2
	S	0.33	137
	R	0.66	68
Labiotermes sp.	W	0.16	282
Macrotermes ukuzii	W.R.S.		
Mastotermes darwiniensis	W	0.00-0.23.47	–2
Nasutitermes corniger	W	6-8	8-6
	S	.9.-28.4	50-2
	Whole colony	27-40.81.68	2-0.5
N. exitiosus	W	0.5.6	-8
Nasutitermes sp.	W	0.20-13.28	226-3
	S	0.87-7.44	52-6
	W+S	1.11.-18.31	41-2
Neocapritermes sp.	W	0	
Reticulotermes flavipes	W	0.05	913
	S	0.02	2,260
Rhynchotermes speratus	W	3.5	904
	S	0.5	90
Trinervitermes trinervoides	W	6.86	7
	S	4.48-4.73	10
	R	0	
Zootermopsis sp.	W+BL	0.06	753

*W, Workers; R, Reproductives S, Soidiers; BL, Branchypterous larvae; TDN, Time required for termites to double their nitrogen content.

c.f. Breznak[24]

that diazotrophic intestinal microflora contribute significantly to their nutritional status.

Cyanobacteria associate with lower animals such as sea squirts.[32] Wilkinson and Fay[166] obtained acetylene reduction by Red Sea sponges carrying symbiotic cyanobacteria but not by similar sponges devoid of such symbionts. Guerinot et al.[59] claimed that psychrophilic diazotrophs in the guts of sea urchin could aceounts for 8-14% of their nitrogen intake.

The most studied association with animals occur among termites. It has been suggested that intestinal diazotrophs might be important in the nitrogen metabolism of insects. Breznak *et al.*[22] demonstrated their presence and activity in the guts of termites. The dominant diazotroph appears to be *Citrobacter freundii*[56] and *Enterobacter agglomeran.*[134]

Leach and Gronovsky[93] suggested that termites can reutilize the nitrozen present in their uric acid excreta either directly or by their symbionts. Uric acid content is about five *per cent* of their dry weight of lower termites.[135] In case of *Reticutoterms flavipes*, 93-95% of the uric acid was stored in the fat body. The increase in the uric acid in *Reticuloterms flavipes* was at the expense of non uric acid nitrogen present in the termite during the uric acid synthesis. The uric acid nitrogen and non-uric acid nitrogen are utilized and degraded by uricase enzyme from gut bacteria, *Streptococcus sp. Bacteriods sp.*, and *Citrobactcr sp.*, In man [160] birds, [9,111] uricolysis has been reported. After series of experiments Mauldin *et al.*[101] concluded that normal gut bacteria and two of the protozoa participate in the synthesis of amino acids synthesis in the cell protein. But Nazarczuk *et al.*[115] observed free amino acids of *N. exitiosus* and *C. lacteus*, contrary to expectation, increased on starvation indicated that amino acids were not mobilized by either the termites or their gut microbiots to provide carbon for growth or maintenance.

Mishra and Sen Sharma[108] reported lignin degradation by hindgut microbiota of sandal wood fed *Neotermes bosei*. ^{14}c labelled cinnamic acid fed wood by *Coptotermes lacteus* and *Nasutitermes exitiosus* proved lignolysis by meauring $^{14}CO_2$ release. In cockroach, microbial involvement in uricolysis is not clearly indentified, but it might be accomplished by gut bacteria or Mycetocyte present in the fat body. A sharp uric acid accumulation on higher nitrogen diets was mobilized when they were shifted to low nitrogen diets. Uricolysis has also been reported from human cells, [160] birds.[9,111]

A number of chitinolytic bacteria in gut have been known in different animals. These bacteria secrete chitinase and chitin serve as the source of nitrogen. Nitrogen fixing bacteria have also been reported from Sea urchins *Strongylocentrotus droebachianses*[59]. Eutick[51] *et al.* and French *et al.*[56] isolated putative dinitrogen fixing organisms *Entrobacter agglomeran* from Australian termites. They were confirmed to fix nitrogen by using N^{15} and N^{13} technique.[137,138]

The gut bacteria supply amino acids to the host termites. Mauldin and Suythe[102] found that amino acid content in the form of protein of *Coptotermes* *formosans* changed little when the termites were partially or completely defaunated and concluded that the termites can maintain their protien levels without protozoa. Speck *et al.*[150] found that removal of the gut bacteria of *Reticulotermes santonensis* led to a decrease in free amino acids but no change was recorded in higher termites-*Nasutitermes nigriceps*. Using streptomycin, some 35% decrease of free amino acid was observed after 18 days of antibiotic treatment. Measurement of the specific activity of the amino acids present in the cellular protein synthesized from C^{14}-labelled acetate showed that completely defaunated termites or termites fed antibiotics were unable to synthesize these amino acids as readily as normal termites or partially defaunated terminates.

Future Perspectives

The metabolism of carbohydrate, protein and other nitrogenous compounds are particularly deserving of research, since the efficiency of energy derivation from various carbon source utilization is probably a determining factor in the efficiency of rumen and associative organisms. Considerable advancement has been made in our understanding of gut microbiota in terms of its community, structure, composition and the roles of microorganisms in corbon, nitrogen, sulfur, and the energy regulation of insects, cattle and man. Our knowledge of these systems is incomplete and fragmented.

The use of dinitrogen by termites as a nitrogen source had been speculated ever since Cleveland's claim that termite could live perhaps indefinitely on a diet of pure cellulose. However, not until recently has this speculation actually received good experimental support. With the acetylene reduction technique N_2 fixation has been inferred for a wide variety of termite species, since nitrogen fixation is a strictly prakaryotic phenomenon, it has been generally assumed that the fixation is mediated by gut bacteria. Clearly more research is needed on this critical question of termite nurition. Likewise, nitrogen fixing bacteria have becn screened from the excreta of guineapigs, pigs, deer, cattles, sheeps, blood of whales and certain human natives of New Guinea whose diet is particularly rich in carbohydrate. A systematic study is required to isolated these bacteria and characterize them both at taxonomic and physiological levels. We still do not know the exact mechanism by which the key intermediate compound after dinitrogen fixation-the 'ammonia' is essimilated into amino acids. The ability of rumen and termite gut bacteria to provide amino acids to their respective host is an important metabolic pathway and needs a critical detailed elucidation. Likewise, lignin and pectin degradation by gut microbiota are intriguing since much of alimentary tract appears anaerobic and the natural mechanisms for enaerobic lignolysis are not known

Methanogenic bacteria grow on hydrogen plus carbondioxide as sole energy source. The enzymes catalysing the reversible activation of molecular hydrogen are called hydrogenases.

The enzyme hydrozenase are wide spread in bacteria, made up of non-sulfur protein. Methanogenic bacteria take up hydrogen *via* an active hydrogenase which is specific for Factor 420 as electron acceptor. This factor functions as electron donor for CO_2 reduction to methane and for CO_2 assimilation into cell carbon. The major unsolved question involved in the research on hydrogenases are, the number of hydrogenase in one organism, their localization, function, sensitivity to oxygen, the iron-sulfur content, the precise mechanism of the hydrogenase reaction and the role of iron-sulfer centre(s) in hydrogen activation and electron transfer.

The very existence of life on this planet depends on the cycling of the major biological elements - C,H,O,N and S - and it is true that microbes play an important part in determining the turnover rate of biological cycles. The biological sulfur cycle is no exception to this generalisation. The bioenergetics of sulfate respiring bacteria, *Desulfovibrio* and *Desulfotomaculum* are quite challenging. The idea of hydrogen cycling as mechanism for energy coupling in these bacteria offers a conceptual frame work within which a number of unique physiological and biochemical observarions concerning these bacteria can be rationalised. A thorough study is needed on the rumen inhabiting sulfur bacteria.

In the last one decade, a considerable body of information on the ultrastructure of termite gut associative microorganisms and also the cattle rumens have been published. Most of these information, however, are related to the study of the morphology of bacteria and ultrastructure of alimentary canal. Detailed study is warranted to understand the close adherence/association between animal and bacterial cell boundaries, their transport biology and also the physiology and biochemistry of various secretive enzyme at structural and regulatory levels.

Human biology, Entomolgy and Microbiology are quite distinct disciplines, and problems important to one are often not appreciated, and seldom entertained by researchers of the other. For fundamental microbiol bio-technologists with basic interest in internal micro-ecology, it might be useful to consider the gut of a xylophagous insects (termite), more seriously as a potential model system. Therein, rests a fertile scientific ground worth cultivating and promote a prospects for a bountiful harvest would seem good.

The task ahead is difficult and will require combined consistent efforts of many disciplines of biological research. The study must involve scientists as well as bacteria, animals and plant. It seems to us that the time is ripe, indeed almost over-ripe, to bring togethet experts from all concerned diciplines in order to attempt a synthesis of current understanding of these intriguing microcoenosis.

Summary

Rumen and the gut microbiologists are basically concerned with the associative microflora which contribute to a great deal in the digestion of food.

Current interest in the rumen ecosystem has arisen principally because most animals of agricultural importance (except pigs) are ruminants or near ruminants. The recent emphasis is on the intensive rearing of animals to raise early and produce maximum dairy and meat products. Modern feeds are very different and bring about digestive disturbance and illness in the animals. A clear understanding on the biology of rumen and its microorganisms will certainly overcome these difficulties and would increase the overall efficiency of rapid digestion in the alimentary tract.

In the last one decade varions cellulolytic bacteria, fungi and actinomycetes have been screened. The complex polysaccharide fibres and pectin digesting microbes have been discovered. Starch containing feed came into prominence with the intensive cattle rearing intraduced and a number of rumen bacteria were implicated to hydrolyze starch, and feed lipids. In addition, microbes have been found to perform proteolysis and deamination. Rumen type microbiol systems also occur in muds and waters and in the anaerobic digesters first used for sewage purification and now projected as a source of biomass energy.

This review article also discusses the role of associative and surface adhesive microorganisms in the metabolism of isoptera, the 'white ants' and nocturnal cockroaches. Symbiotic microorganisms comprising of bacteria and fungi (higher and lower termites) and protozoa (lower termites) are in the hindgut. The end products of protozoal and bacterial fermentations are thought to be used by termites an energy sources.

There are several reports on the presence of diazotrophic bacteria from the excreta of humans, guineapigs, sheep, deer and the hindgut of termites. The dominant diazotrophs appear to be *Citrobacter freundii* and *Enterobacter agglomerans*.

Hydrogen plays and integral role in anaerobic food chain mediated by several fermentative bacteria. Many of these rumen and gut bacteria take advantage of the 'hydrogen valve' the system necessary to release excess of reducing power in the form of gaseous hydrogen into the environment in order to obtain extra energy for substrate level phosphorylation. Other bacteria consume hydrogen as electron donor for energy generation and/or as hydrogen donor for reductive biosynthetic reactions. Methane and carbondioxide are in major proportion of the atmospheric trace gases. Since a great part of methane is produced from hydrogen and carbon dioxide primarily by methanogenic bacteria; the role of hydrogen as an intermediate in the cycle of organic matter becomes evident.

Sulfur is found in living organisms in several oxidation-reduction states from the highly oxidized sulfate as in polysaccharide sulfate, to the completely reduced form, as in thiol groups of amino acids and proteins. Microbes contribute to the sulfur cycle to a great deal. Sulfate is reduced by microorganisms in two main ways: assimilation, where sulfate is converted to the thio level and incorporated into amino acids and proteins, dissimilation, where sulfate reducing bacteria tolerate some of the most extreme terrestrial physical and chemical stress and generate large amount of H_2S. Higher animals, especially mammals, can not reduce sulfate to the thiol level so that they are dependent on an exogenous source of these compounds in organic form such as cysteine (cystine) and methionine. The reduction of sulfate in ruminants is associated with the microflora in the gut. Little is known about the sulfur metabolism of invertebrates. The fruit fly, *Drosophila melanogaster*; requires cysteine and methionine for its growth and development in defined media. Role of other elements like Copper, Molybdenum, Nickel, Selenium and Zinc has also been discussed.

The basic purpose of this review is to update our information on the alimentary tract and gut microbiota with special emphasis on its nutritional physiology and regulatory mechanisms published in the last ten years.

Acknowledgements

The genesis of this review article goes back to the encouraging discussion in 1977 between the senior author (AV) and Professor P.N. Srivastava, Vice Chancellor, Jawaharlal Nehru University, then Dean, School of Life Sciences on the physiology of cellulose digestion by members of the isoptera. Since then our microbiology laboratory is actively engaged on the understanding the physiology of associative microorganisms in termite gut and soil mound around them. Authors are thankful to Professor C.A. Reddy, Michigan State University, USA and Professor H.D. Peck, Jr, Georgia University, USA for helpful discussions. Thoughtful criticisms were received from Professor R. Thauer, West Germany. Partial financial assistance was obtained from India Council of Agricultural Research, New Delhi to do research work on the termite associative microorganisms.

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